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(54) KNOCKOUT NON-HUMAN ANIMAL

(57)Abstract:
PROBLEM TO BE SOLVED: To develop a homologous recombination method for simply and rapidly producing a knockout non-human animal in which a blood level of an adiposity promoting protein and/or an expression level itself are changed or the adiposity promoting protein is eliminated by destroying a part or all of the genomic gene of the adiposity promoting protein, and to provide the knockout non-human animal produced by using the homologous recombination method.
SOLUTION: In this non-human animal or a part thereof comprising an individual of the knockout non-human animal, a descendant animal or the part thereof, all or one of allyls in a diploid of the genomic gene of the adiposity promoting protein having a ≥ about 6,000 molecular weight are substituted with dysfunctional mutant genes, or all or one of the allyls in the diploid of the genomic gene are destroyed so that their functions are deleted. A method for evaluating glucolipid metabolism control ability is provided by using the non-human animal or the part thereof.

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CLAIMS

[Claim(s)]
[Claim 1]
The nonhuman animal characterized by being destroyed and becoming so that one [all in the diplont of the genome gene of the adiposity acceleration protein which is an individual, its descendant animal, or those some of knockout nonhuman animal, and is about 6000 or more molecular weight, or] allyl compound may be permuted by the functional deletion mold mutant alle or one [all in the diplont of said genome gene or] allyl compound may carry out deletion of the function, or its part.
[Claim 2]
The nonhuman animal according to claim 1 characterized by the adiposity acceleration protein which is about 6000 or more molecular weight being the protein which consists of one of the following amino acid sequences, and has adiposity acceleration activity, or its part.
<Amino acid sequence>
(a) The amino acid sequence shown by the array number 1,
(b), and 1 or two or more amino acid are deletion and the amino acid sequence added or permuted, [the amino acid sequence shown by the array number 1]
(c) The partial amino acid sequence in which 26 amino acid comes to carry out deletion from the amino terminus in the amino acid sequence shown by the array number 1,
(d) The amino acid sequence which comes to add a methionine to the amino terminus in the amino acid sequence of the above (c),
(e) The amino acid sequence shown by the array number 1, and the amino acid sequence which has 80% or more of array identity,
(f) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th,

(g) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and DNA which has the base sequence which has 80% or more of array identity,
(h) The amino acid sequence in which a code is carried out by DNA which consists of a base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and a base sequence which has a complementarity, and DNA hybridized under stringent conditions
(i) Amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the array number 2;
[Claim 3]
An individual, its descendant animal, or those some of knockout nonhuman animal characterized by being destroyed and becoming so that one [all in the diplont of the genome gene of the protein which consists of an amino acid sequence shown by the array number 1, or] allyl compound may be permuted by the functional deletion mold mutant alle or one [all in the diplont of said genome gene or] allyl compound may carry out deletion of the function
[Claim 4]
The genome gene of the protein which consists of an amino acid sequence shown by the array number 1 Between the base numbers 57 and 58 on the base sequence shown by the array number 2 (between exons 1 and 2), Between the base numbers 232 and 233 (between exons 2 and 3), between the base numbers 318 and 319 (between exons 3 and 4), The nonhuman animal according to claim 3 characterized by being what contains the intron between the base numbers 447 and 448 (between exons 4 and 5) in the part corresponding to between the base numbers 506 and 507 (between exons 5 and 6), or its part.
[Claim 5]
The nonhuman animal according to claim 3 to which the genome gene of the protein which consists of an amino acid sequence shown by the array number 1 is characterized by the permutation of a part of [at least] DNA in the structural protein coding region of the gene concerned, deletion, and/or addition coming to be destroyed, or its part.
[Claim 6]
The nonhuman animal according to claim 5 which a part of [at least] DNA in a structural protein coding region is the bases in an exon 3 and 4 fields, and is characterized by deletion coming to destroy the base concerned, or its part.
[Claim 7]
The approach destroyed so that the genome gene of the adiposity

acceleration protein which is about 6000 or more molecular weight may be permuted by the functional deletion mold mutant alle or said genome gene may carry out deletion of the function The gene cassette which has the selective marker gene by which promoterregion is removed is contained. And the selective marker gene promotor loess recombination vector which has a homologous base sequence is used for a part of DNA of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight. The nonhuman animal according to claim 1 characterized by being a part or all of the vector concerned, and being the approach of coming to permute a part of [at least] DNA in the structural protein coding region of said genome gene by DNA containing said gene cassette, or its part.

[Claim 8]

A gene cassette SA of the mouse EN2 origin (splicing accepter), IRES of the encephalomyocarditisecond Virus origin (internal ribosome entry site), pA (polyadenylation signal) of the SV40 origin is flush-end--ization-processed to betageo with which it makes it come to unite beta-galactocidase and a neomycin resistance gene. The nonhuman animal according to claim 7 characterized by being becoming DNA (SA-IRIS-beta geo-pA gene cassette), or its part.

[Claim 9]

The nonhuman animal according to claim 7 or 8 characterized by a part of [at least] DNA in a structural protein coding region being the bases in an exon 3 and 4 fields, or its part.

[Claim 10]

The nonhuman animal characterized by coming to decrease rather than the amount of the said gene expression in a wild type nonhuman animal with the amount of adiposity acceleration protein gene expression of the same kind which is an individual, its descendant animal, or those some of knockout nonhuman animal, and are about 6000 or more molecular weight, or its part.

[Claim 11]

The nonhuman animal according to claim 10 characterized by the adiposity acceleration protein which is about 6000 or more molecular weight being the protein which consists of one of the following amino acid sequences, and has adiposity acceleration activity, or its part.

<Amino acid sequence>

(a) The amino acid sequence shown by the array number 1,

(b), and 1 or two or more amino acid are deletion and the amino acid sequence added or permuted, [the amino acid sequence shown by the array number 1]

(c) The partial amino acid sequence in which 26 amino acid comes to carry out deletion from the amino terminus in the amino acid sequence shown by the array number 1,

(d) The amino acid sequence which comes to add a methionine to the amino terminus in the amino acid sequence of the above (c),
(e) The amino acid sequence shown by the array number 1, and the amino acid sequence which has 80% or more of array identity,
(f) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th,
(g) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and DNA which has the base sequence which has 80% or more of array identity,
(h) The amino acid sequence in which a code is carried out by DNA which consists of a base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and a base sequence which has a complementarity, and DNA hybridized under stringent conditions
(i) Amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the array number 2;
[Claim 12]
Claims 1-11 characterized by expressing 1 or two or more properties which are chosen from the group of the following (a) - (e) are the nonhuman animal of a publication, or its part either.
(a) Usually, breeding or breeding with high-calorie-diet food shows a hyperglycemia value as compared with a wild type nonhuman animal.
(b) Usually, breeding or breeding with high-calorie-diet food shows glucose tolerance lowering as compared with a wild type nonhuman animal.
(c) Breeding with high-calorie-diet food shows the amount of high blood cholesterol levels as compared with a wild type nonhuman animal.
(d) Breeding with high-calorie-diet food shows the amount of high triglyceride in liver as compared with a wild type nonhuman animal.
(e) Change the amount of manifestations of a glycolipid metabolic turnover related factor as compared with a wild type nonhuman animal.
[Claim 13]
Claims 1-12 characterized by a nonhuman animal being a mouse are the nonhuman animal of a publication, or its part either.
[Claim 14]
Claims 1-13 to which some of individuals of a nonhuman animal, its descendant animals, or nonhuman animals in those parts are characterized by being the organization or cell of the nonhuman animal origin are the nonhuman animal of a publication, or its part either.
[Claim 15]
The totipotency cell characterized by being destroyed and becoming so that

one [all in the diplont of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight, or] allyl compound may be permuted by the functional deletion mold mutant alle and one [all in the diplont of ** or said genome gene or] allyl compound may carry out deletion of the function.

[Claim 16]

The totipotency cell according to claim 15 characterized by being destroyed and becoming so that the genome gene of the protein which consists of an amino acid sequence shown by the array number 1 may be permuted by the functional deletion mold mutant alle or said genome gene may carry out deletion of the function.

[Claim 17]

The model animal of the disease accompanied by a glycolipid metabolic turnover malfunction which consists of a nonhuman animal according to claim 1 to 14.

[Claim 18]

The symptoms analysis approach of a disease that the adiposity acceleration protein which is characterized by conducting the growth differentiation in the period of from fetus until fatal, development, observation of living activities, pathology histological inspection, or biochemical inspection about a nonhuman animal or its part according to claim 1 to 14 and which is about 6000 or more molecular weight involves.

[Claim 19]

The symptoms analysis approach according to claim 18 that the disease in which the adiposity acceleration protein which is about 6000 or more molecular weight participates is a disease accompanied by a glycolipid metabolic turnover malfunction.

[Claim 20]

It is the assessment approach of glycolipid metabolic regulation capacity,

(1) Claims 1-14 are the first process which contacts an examined substance to the nonhuman animal of a publication, or its part either,

(2) The second process [contrast / measure the index value which has a correlation in the amount of manifestations or the amount concerned of the adiposity acceleration protein which is about 6000 or more molecular weight in the nonhuman animal to which said examined substance was contacted, or its part, and],

The third process which evaluates the glycolipid metabolic regulation capacity of an examined substance based on the comparison result of (3) and (2)

The assessment approach characterized by ****(ing).

[Claim 21]

The assessment approach according to claim 20 that glycolipid metabolic

regulation capacity is a cholesterol production controllability.
[Claim 22]
The assessment approach according to claim 20 that glycolipid metabolic regulation capacity is biosynthesis of fatty acid and/or fatty-acid metabolic regulation capacity.
[Claim 23]
The assessment approach according to claim 20 that glycolipid metabolic regulation capacity is sugar metabolic regulation capacity.
[Claim 24]
The assessment approach according to claim 20 that glycolipid metabolic regulation capacity is a glycolipid metabolic turnover related factor manifestation controllability.
[Claim 25]
The assessment approach according to claim 24 which is 1 or two or more factors which are chosen from the group which a glycolipid metabolic turnover related factor becomes from PEPCK, G6P, UCP1, and FAS and ACO.
[Claim 26]
Claims 20-25 whose index values which have a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight are the amount of manifestations of a glycolipid metabolic turnover related factor are the assessment approaches of a publication either.
[Claim 27]
The assessment approach according to claim 26 which is 1 or two or more factors which are chosen from the group which a glycolipid metabolic turnover related factor becomes from PEPCK, G6P, UCP1, and FAS and ACO.
[Claim 28]
Claims 20-25 whose index values which have a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight are the blood sugar level are the assessment approaches of a publication either.
[Claim 29]
Claims 20-25 whose index values which have a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight are glucose tolerance are the assessment approaches of a publication either.
[Claim 30]
Claims 20-25 whose index values which have a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight are the amount of blood cholesterol levels are the

assessment approaches of a publication either.
[Claim 31]
Claims 20-25 whose index values which have a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight are the amount of triglyceride (neutral fat) are the assessment approaches of a publication either.
[Claim 32]
Claims 20-31 are the screening approaches of the glycolipid metabolic regulation matter characterized by selecting the examined substance which has glycolipid metabolic regulation capacity based on the glycolipid metabolic regulation capacity evaluated by the assessment approach of a publication either.
[Claim 33]
The therapy agent or preventive of a disease accompanied by the glycolipid metabolic turnover malfunction obtained by the screening approach according to claim 32.
[Claim 34]
The therapy agent according to claim 33 or preventive whose disease accompanied by a glycolipid metabolic turnover malfunction is glucose tolerance lowering, diabetes mellitus, hyperlipidemia, hypertension, arteriosclerosis, a coronary artery disease, angina pectoris, myocardial infarction, or cardiovascular disturbance.
[Claim 35]
either of claims 1-14 -- the selective marker gene promotor loess recombination vector which contains DNA (gene cassette) which has the selective marker gene by which the promoterregion for creating the nonhuman animal, its part, or the totipotency cell according to claim 15 or 16 of a publication is removed, and has a base sequence [homologous / DNA / a part of / of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight].
[Claim 36]
either of claims 1-14 -- the nonhuman animal of a publication, or its part -- or SA of the mouse EN2 origin for creating a totipotency cell according to claim 15 or 16 (splicing accepter), IRES of the encephalomyocarditisecond Virus origin (internal ribosome entry site), DNA (SA-IRIS-betageo-pA gene cassette) which comes to process pA (polyadenylation signal) of the SV40 origin to betageo with which it makes it come to unite beta-galactocidase and a neomycin resistance gene flush end-ization is contained. And the selective marker gene promotor loess recombination vector which has a base sequence [homologous / DNA / a part of / of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight].

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[Field of the Invention]
[0001]
This invention relates to a knockout nonhuman animal etc. This invention is an individual, its descendant animal, or those some of knockout nonhuman animal in more detail. It being destroyed and becoming so that one [all in the diplont of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight, or] allyl compound may be permuted by the functional deletion mold mutant alle or one [all in the diplont of said genome gene or] allyl compound may carry out deletion of the function It is related with totipotency cells, such as an embryonic stem cell indispensable to creation of the nonhuman animal by which it is characterized or its part, the nonhuman animal concerned, or its part, those utilization, etc. This invention is useful in fields, such as drugs development to the disease accompanied by a glycolipid metabolic turnover malfunction etc.
[Background of the Invention]
[0002]
The same applicant for a patent indicated DNA which consists of a base sequence which carries out the code of the amino acid sequence of the protein which consists of an amino acid sequence shown in the description of JP,2000-356637,A by the adiposity acceleration protein which is about 6000 or more molecular weight, especially the array number 1, and has adiposity acceleration activity. The disclosure concerned is related with the analytical method of the amount of depot fat in the fat cell using the protein which consists of an amino acid sequence shown by the adiposity acceleration protein which is about 6000 or more molecular weight, especially the array number 1, and has adiposity acceleration activity etc., and makes easy utilization of the adiposity acceleration protein concerned, analysis in a molecular level, etc. (see the patent reference 1 and 2). However, it cannot necessarily be said that the laboratory animal which can analyze the utilization and physiological meaning of the adiposity acceleration protein concerned in mammalian individual level in a detail is fully established.
The concentration in blood of the protein which consists of an amino acid sequence shown in more detail by the adiposity acceleration protein which is about

6000 or more molecular weight until now, especially the array number 1, and has adiposity acceleration activity is in the intraperitoneal fat tissue area value of the abdomen cross section, and a forward correlation, and it is found out that the amount of intraperitoneal fat tissues can be calculated from the concentration of the adiposity acceleration protein concerned. It is thought from the experiment in a molecular level, cell level, etc. that the adiposity acceleration protein concerned is one of the factors in connection with visceral fat (are recording) syndrome.
As a laboratory animal which can analyze the utilization and physiological meaning of other adiposity acceleration protein in mammalian individual level in a detail The DGAT gene disruption mouse, the 11 beta-HSD 1 gene-disruption mouse, etc. are known. In these laboratory animals The gene made into the object on the chromosome of totipotency cells, such as an embryonic stem cell of an animal which performs a gene alteration, first, the POJINEGA method (For example, the targeting vector (selective marker gene promotor content recombination vector) in which a part of object gene on a chromosome has the promoterregion of a selective marker gene is used.) It destroys by the approach [**** / the homonous rearranging method which transposes a part of object gene on a chromosome to a targeting vector]. After making this mix in the blastocyst of a parent animal, it has succeeded in falling or vanishing the concentration of the adiposity acceleration protein [in the living body] concerned by creating the chimera primitive mouse which is ** to which made continue embryonic differentiation and it was made to give birth.
On the other hand, each of PEPCK (phosphoenolpyruvae carboxykinase), G6P (G6 Pase;glucose-6-phosphatase), UCP(s)1 (uncoupling protein 1), and FAS (fatty acid synthase) and ACO (acylCoA oxidase) are well-known as protein relevant to a glycolipid metabolic turnover (on these descriptions, a glycolipid metabolic turnover related factor may be called collectively below).
Specifically, it is known that PEPCK (phosphoenolpyruvae carboxykinase) and G6P (G6 Pase;glucose-6-phosphatase) are enzymes which carry out the catalyst of two steps of the reactions of the glyconeogenesis which produces a glucose from a pyruvic acid in a liver cell, respectively. It is known that UCP1 (uncoupling protein 1) is protein which promotes the ATP production from free fatty acid. being FAS (fatty acid synthase) -- it is known that it is a fatty-acid biosynthesis. It is known that ACO (acylCoA oxidase) is a fatty-acid metabolic turnover enzyme. However, the relation between glycolipid metabolic turnovers or these glycolipid metabolic turnover related factors, and the adiposity acceleration protein that is about 6000 or more molecular weight was not known.
[Patent reference 1] JP,2000-356637,A
[Patent reference 2] The international disclosure pamphlet 02nd / No. 10772
[Description of the Invention]
[Problem(s) to be Solved by the Invention]
[0003]
The detailed functional analyses (namely, detailed analysis about the physiological function which the adiposity acceleration protein concerned exerts on mammalian by comparing a knockout nonhuman animal with a wild type nonhuman animal etc.)

of the adiposity acceleration protein concerned in individual level are becoming important especially under such a situation. Then, a part or creation of the knockout nonhuman animal which it destroyed [animal] altogether, and changed the level in blood and/or the manifestation level itself of the adiposity acceleration protein concerned, or vanished the adiposity acceleration protein concerned was tried for the genome gene of the adiposity acceleration protein concerned by the homonous rearranging method.
This invention is made in view of the situation as above, and makes the technical problem development of a part of genome gene of the adiposity acceleration protein concerned for creating simple and promptly offering the above knockout nonhuman animals and the knockout nonhuman animal concerned, or the homonous recombination approach aiming at all destruction etc.
[Means for Solving the Problem]
[0004]
In the genome gene alteration of the adiposity acceleration protein which is about 6000 or more molecular weight as a result of this invention persons' inquiring wholeheartedly also as that of this situation The gene made into the object on the chromosome of totipotency cells, such as an embryonic stem cell of an animal which performs the genome gene alteration concerned, the promoter trap method (For example, the targeting vector (selective marker gene promotor loess recombination vector) which removed the promoterregion of a selective marker gene) is used. After destroying by the homonous rearranging method which transposes a part of object gene on a chromosome to a targeting vector and making this mix in the blastocyst of a parent animal, it became possible to create the chimera primitive mouse which is ** to which made continue embryonic differentiation and it was made to give birth, and the effective thing was found out.

Furthermore, as a result of inquiring wholeheartedly using the descendant (said chimera primitive mouse and its descendant may be called this invention knockout mouse in this description below) who said chimera primitive mouse was bred and created, in this invention knockout mouse, compared with a wild type mouse, the blood sugar level at the time of hungry is rising, and glucose tolerance lowering is seen further, namely, it turned out that insulin susceptibility falls. Moreover, in this invention knockout mouse, since the amount of triglyceride in the liver at the time of a high fat and high sucrose foods intake also increased intentionally, it found out that this animal could serve as a model mouse showing the symptoms similar to the abnormalities in a glycolipid metabolic regulation.
Furthermore, this invention knockout-mouse origin organization is used, and it is a glycolipid metabolic turnover related factor :P EPCK gene, The result of having examined many things about the manifestation of G6P gene, UCP1 gene, an FAS gene, or an ACO gene, (1) FAS [which is the manifestation of UCP1 and fatty-acid biosynthesis enzyme which PEPCK which is the enzyme which promotes a glyconeogenesis in a liver cell, and the manifestation of G6P go up, and rise energy (ATP) production in;(2) fat (brown or white) cell], In the liver cell and fat cell which are bearing important work in the glycolipid metabolic turnover since

the manifestation of ACO which is furthermore a fatty-acid metabolic turnover enzyme went up It turned out that the adiposity acceleration protein which is about 6000 or more molecular weight of this invention has the operation in connection with manifestation control of these glycolipid metabolic turnover related factors. Therefore, when the knockout nonhuman animal of this invention or its part was used as a model animal, such gene expression found out becoming a useful index, in order to screen the matter which has glycolipid metabolic regulation capacity.

This invention comes to be completed based on the above-mentioned knowledge. Namely, this invention,

[1] The nonhuman animal characterized by being destroyed and becoming so that one [all in the diplont of the genome gene of the adiposity acceleration protein which is an individual, its descendant animal, or those some of knockout nonhuman animal, and is about 6000 or more molecular weight, or] allyl compound may be permuted by the functional deletion mold mutant alle or one [all in the diplont of said genome gene or] allyl compound may carry out deletion of the function, or its part;

[2] A nonhuman animal or its part of [1] publication characterized by the adiposity acceleration protein which is about 6000 or more molecular weight being the protein which consists of one of the following amino acid sequences, and has adiposity acceleration activity;

<Amino acid sequence>

(a) The amino acid sequence shown by the array number 1,

(b), and 1 or two or more amino acid are deletion and the amino acid sequence added or permuted, [the amino acid sequence shown by the array number 1]

(c) The partial amino acid sequence in which 26 amino acid comes to carry out deletion from the amino terminus in the amino acid sequence shown by the array number 1,

(d) The amino acid sequence which comes to add a methionine to the amino terminus in the amino acid sequence of the above (c),

(e) The amino acid sequence shown by the array number 1, and the amino acid sequence which has 80% or more of array identity,

(f) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th,

(g) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and DNA which has the base sequence which has 80% or more of array identity,

(h) The amino acid sequence in which a code is carried out by DNA which consists of a base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and a base sequence which has a complementarity, and DNA hybridized under stringent conditions

(i) Amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the array number 2;

[3] An individual, its descendant animal, or those some of knockout nonhuman animal characterized by being destroyed and becoming so that one [all in the diplont of the genome gene of the protein which consists of an amino acid sequence shown by the array number 1, or] allyl compound may be permuted by the functional deletion mold mutant alle or one [all in the diplont of said genome gene or] allyl compound may carry out deletion of the function;
[4] The genome gene of the protein which consists of an amino acid sequence shown by the array number 1 Between the base numbers 57 and 58 on the base sequence shown by the array number 2 (between exons 1 and 2), Between the base numbers 232 and 233 (between exons 2 and 3), between the base numbers 318 and 319 (between exons 3 and 4), A nonhuman animal or its part of [3] publications characterized by being what contains the intron between the base numbers 447 and 448 (between exons 4 and 5) in the part corresponding to between the base numbers 506 and 507 (between exons 5 and 6);
[5] A nonhuman animal or its part of [3] publications to which the genome gene of the protein which consists of an amino acid sequence shown by the array number 1 is characterized by the permutation of a part of [at least] DNA in the structural protein coding region of the gene concerned, deletion, and/or addition coming to be destroyed;
[6] A nonhuman animal or its part of [5] publications which a part of [at least] DNA in a structural protein coding region is the bases in an exon 3 and 4 fields, and are characterized by deletion coming to destroy the base concerned;
[7] The approach destroyed so that the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight may be permuted by the functional deletion mold mutant alle or said genome gene may carry out deletion of the function The gene cassette which has the selective marker gene by which promoterregion is removed is contained. And the selective marker gene promotor loess recombination vector which has a homologous base sequence is used for a part of DNA of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight. A nonhuman animal or its part of [1] publication characterized by being a part or all of the vector concerned, and being the approach of coming to permute a part of [at least] DNA in the structural protein coding region of said genome gene by DNA containing said gene cassette;
A gene cassette [8] SA of the mouse EN2 origin (splicing accepter), IRES of the encephalomyocarditisecond Virus origin (internal ribosome entry site), pA (polyadenylation signal) of the SV40 origin is flush-end--ization-processed to betageo with which it makes it come to unite beta-galactocidase and a neomycin resistance gene. A nonhuman animal or its part of [7] publications characterized by being becoming DNA (SA-IRIS-beta geo-pA gene cassette);
[9] A nonhuman animal or its part of [7] characterized by a part of [at least] DNA in a structural protein coding region being the bases in an exon 3 and 4 fields, or [8] publications;
[10] The nonhuman animal characterized by coming to decrease rather than the amount of the said gene expression in a wild type nonhuman animal with the amount of adiposity acceleration protein gene expression of the same kind which is

an individual, its descendant animal, or those some of knockout nonhuman animal, and are about 6000 or more molecular weight, or its part;
[11] A nonhuman animal or its part of [10] publications characterized by the adiposity acceleration protein which is about 6000 or more molecular weight being the protein which consists of one of the following amino acid sequences, and has adiposity acceleration activity;
<Amino acid sequence>
(a) The amino acid sequence shown by the array number 1,
(b), and 1 or two or more amino acid are deletion and the amino acid sequence added or permuted, [the amino acid sequence shown by the array number 1]
(c) The partial amino acid sequence in which 26 amino acid comes to carry out deletion from the amino terminus in the amino acid sequence shown by the array number 1,
(d) The amino acid sequence which comes to add a methionine to the amino terminus in the amino acid sequence of the above (c),
(e) The amino acid sequence shown by the array number 1, and the amino acid sequence which has 80% or more of array identity,
(f) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th,
(g) The amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and DNA which has the base sequence which has 80% or more of array identity,
(h) The amino acid sequence in which a code is carried out by DNA which consists of a base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and a base sequence which has a complementarity, and DNA hybridized under stringent conditions
(i) Amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the array number 2;
[12] [1]- [11] characterized by expressing 1 or two or more properties which are chosen from the group of the following (a) - (e) is the nonhuman animal of a publication, or its part either.;
(a) Usually, breeding or breeding with high-calorie-diet food shows a hyperglycemia value as compared with a wild type nonhuman animal.
(b) By breeding with standard foods or high-calorie-diet food, glucose tolerance falls as compared with a wild type nonhuman animal.
(c) Breeding with high-calorie-diet food shows the amount of high blood cholesterol levels as compared with a wild type nonhuman animal.
(d) Breeding with high-calorie-diet food shows the amount of high triglyceride in liver as compared with a wild type nonhuman animal.
(e) Change the amount of manifestations of a glycolipid metabolic turnover related factor as compared with a wild type nonhuman animal.
[13] [1]- [12] characterized by a nonhuman animal being a mouse is the nonhuman animal of a publication, or its part either.;

[14] [1]- [13] to which some of individuals of a nonhuman animal, its descendant animals, or nonhuman animals in those parts are characterized by being the organization or cell of the nonhuman animal origin is the nonhuman animal of a publication, or its part either.;
[15] Totipotency cell characterized by being destroyed and becoming so that one [all in the diplont of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight, or] allyl compound may be permuted by the functional deletion mold mutant alle and one [all in the diplont of ** or said genome gene or] allyl compound may carry out deletion of the function;
[16] Totipotency cell given in [15] characterized by being destroyed and becoming so that the genome gene of the protein which consists of an amino acid sequence shown by the array number 1 may be permuted by the functional deletion mold mutant alle or said genome gene may carry out deletion of the function;
[17] [1] Model animal of the disease accompanied by a glycolipid metabolic turnover malfunction which consists of a nonhuman animal given in - [14];
[18] [1] The symptoms analysis approach of a disease that the adiposity acceleration protein which is characterized by performing the growth differentiation in the period of from fetus until fatal, development, observation of living activities, pathology histological inspection, or biochemical inspection to - [14] about the nonhuman animal of a publication or its part and which is about 6000 or more molecular weight involves;
[19] The symptoms analysis approach given in [18] whose disease in which the adiposity acceleration protein which is about 6000 or more molecular weight participates is a disease accompanied by a glycolipid metabolic turnover malfunction;
[20] It is the assessment approach of glycolipid metabolic regulation capacity,
(1) Claims 1-14 are the first process which contacts an examined substance to the nonhuman animal of a publication, or its part either,
(2) The second process [contrast / measure the index value which has a correlation in the amount of manifestations or the amount concerned of the adiposity acceleration protein which is about 6000 or more molecular weight in the nonhuman animal to which said examined substance was contacted, or its part, and],
The third process which evaluates the glycolipid metabolic regulation capacity of an examined substance based on the comparison result of (3) and (2)
The assessment approach characterized by ****(ing);
[21] The assessment approach given in [20] whose glycolipid metabolic regulation capacity is a cholesterol production controllability;
[22] The assessment approach given in [20] whose glycolipid metabolic regulation capacity is biosynthesis of fatty acid and/or fatty-acid metabolic regulation capacity;
[23] The assessment approach given in [20] whose glycolipid metabolic regulation capacity is sugar metabolic regulation capacity;
[24] The assessment approach given in [20] whose glycolipid metabolic regulation capacity is a glycolipid metabolic turnover related factor manifestation

controllability;
[25] The assessment approach given in [24] which is 1 or two or more factors which are chosen from the group which a glycolipid metabolic turnover related factor becomes from PEPCK, G6P, UCP1, and FAS and ACO;
[26] [20]- [25] whose index value which has a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight is the amount of manifestations of a glycolipid metabolic turnover related factor is the assessment approach of a publication either.;
[27] The assessment approach given in [26] which is 1 or two or more factors which are chosen from the group which a glycolipid metabolic turnover related factor becomes from PEPCK, G6P, UCP1, and FAS and ACO;
[28] [20]- [25] whose index value which has a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight is the blood sugar level is the assessment approach of a publication either.;
[29] [20]- [25] whose index value which has a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight is glucose tolerance is the assessment approach of a publication either.;
[30] [20]- [25] whose index value which has a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight is the amount of blood cholesterol levels is the assessment approach of a publication either.;
[31] [20]- [25] whose index value which has a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight is the amount of triglyceride (neutral fat) is the assessment approach of a publication either.;
[32] [20] - [31] is the screening approach of the glycolipid metabolic regulation matter characterized by selecting the examined substance which has glycolipid metabolic regulation capacity based on the glycolipid metabolic regulation capacity evaluated by the assessment approach of a publication either.;
[33] The therapy agent or preventive of a disease accompanied by the glycolipid metabolic turnover malfunction obtained by [32] by the screening approach of a publication;
[34] The therapy agent or preventive given in [33] whose disease accompanied by a glycolipid metabolic turnover malfunction is glucose tolerance lowering, diabetes mellitus, hyperlipidemia, hypertension, arteriosclerosis, a coronary artery disease, angina pectoris, myocardial infarction, or cardiovascular disturbance;
[35] [1] -- either of - [14] -- selective marker gene promotor loess recombination vector; which contains DNA (gene cassette) which has the selective marker gene by which the promoterregion for creating a totipotency cell the nonhuman animal of a publication, its part, [15], or given in [16] is removed, and has a base sequence [homologous / DNA / a part of / of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight]
[36] [1] -- either of - [14] -- the nonhuman animal of a publication, or its part --

or [15] Or SA of the mouse EN2 origin for creating a totipotency cell given in [16] (splicing accepter), IRES of the encephalomyocarditisecond Virus origin (internal ribosome entry site), DNA (SA-IRIS-betageo-pA gene cassette) which comes to process pA (polyadenylation signal) of the SV40 origin to betageo with which it makes it come to unite beta-galactocidase and a neomycin resistance gene flush end-ization is contained. And selective marker gene promotor loess recombination vector which has a base sequence [homologous / DNA / a part of / of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight];
** is offered.
[Effect of the Invention]
[0005]
By this invention, it is an individual, its descendant animal, or those some of knockout nonhuman animal. It being destroyed and becoming so that one [all in the diplont of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight, or] allyl compound may be permuted by the functional deletion mold mutant alle or one [all in the diplont of said genome gene or] allyl compound may carry out deletion of the function Offer of totipotency cells, such as an embryonic stem cell indispensable to creation of the nonhuman animal by which it is characterized or its part, the nonhuman animal concerned, or its part, and those utilization was enabled. Moreover, it became possible to offer the approach of screening the matter which has glycolipid metabolic regulation capacity useful as the therapy agent or preventive of the disease relevant to glycolipid metabolic turnovers, such as glucose tolerance lowering, diabetes mellitus, hyperlipidemia, hypertension, arteriosclerosis, a coronary artery disease, angina pectoris, myocardial infarction, or cardiovascular disturbance.
[Best Mode of Carrying Out the Invention]
[0006]
This invention is explained below at a detail.
The knockout nonhuman animal in this invention means the nonhuman animal currently destroyed by the condition that all or one side of the allyl compounds (allele) in one locus which exists in the cell of the nonhuman animal containing a germ cell line cell in the living body does not demonstrate the original function. It is destroyed and the knockout nonhuman animal which this invention offers becomes so that the adiposity acceleration protein all of the genome genes of the adiposity acceleration protein which is about 6000 or more molecular weight in one locus on the intracellular chromosome which constitutes a nonhuman animal living body, or one allyl compounds of whose are about 6000 or more activity **** molecular weight may not be discovered.
The genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight in this invention is a field on the nonhuman animal chromosome by which the intron corresponding to mRNA which has the base sequence which carries out the code of the amino acid sequence of the adiposity acceleration protein which is about 6000 or more molecular weight has been included according to the above-mentioned definition, and is not limited to specific

structure, for example, a specific base sequence or intron-exon structure.
[0007]
As adiposity acceleration protein which is about 6000 or more molecular weight in this invention For example, it sets to the protein which consists of an amino acid sequence shown by (a) array number 1, and the amino acid sequence shown by (b) array number 1. In the protein which 1 or two or more amino acid consist of deletion and an amino acid sequence added or permuted, and has adiposity acceleration activity, and the amino acid sequence shown by (c) array number 1 The protein which consists of a partial amino acid sequence to which deletion of the 26 amino acid was carried out from the amino terminus In the amino acid sequence of (namely, the protein which consists of an amino acid sequence shown from the amino acid from the 27th in the amino acid sequence shown by the array number 1 to the 491st), and (d) above (c) The protein which consists of an amino acid sequence by which the methionine was added to the amino terminus (Namely, the protein which consists of an amino acid sequence by which the methionine was added to the amino terminus of the amino acid sequence shown from the amino acid from the 27th in the amino acid sequence shown by the array number 1 to the 491st), (e) It consists of an amino acid sequence shown by the array number 1, and an amino acid sequence which has 80% or more of array identity. And the protein which consists of an amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the protein which has adiposity acceleration activity, and the base sequence shown by (f) array number 2 to the 1476th, (g) It consists of an amino acid sequence in which a code is carried out by DNA which has the base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, and DNA which has the base sequence which has 80% or more of array identity. And DNA which consists of a base sequence shown by the nucleotide from the 1st nucleotide in the protein which has adiposity acceleration activity, and the base sequence shown by (h) array number 2 to the 1476th, and a base sequence which has a complementarity, It consists of an amino acid sequence in which a code is carried out by DNA hybridized under stringent conditions. And the protein which has adiposity acceleration activity, the protein which consists of an amino acid sequence in which a code is carried out by DNA which has the base sequence shown by (i) array number 2 can be mentioned.
Here, the variation naturally produced according to the specific difference of the living thing in which processing which the protein which has the amino acid sequence shown by the array number 1 receives by intracellular, and this protein originate, individual difference, the difference during an organization, etc., the variation of artificial amino acid, etc. are included in "80% or more of array identity" in "the deletion of amino acid, the addition or the permutation" and the above (e) which exist above (b), and (g).
the field which performs artificially "the deletion, the addition, or the permutation" (it may be hereafter described as the alteration of amino acid generally) of amino acid which exists above (b) -- an interlude -- the technique of performing [technique] site-specific mutation installation of common use to DNA which

carries out the code of the amino acid sequence shown by the array number 1 as a method, for example, and making this DNA discover with a conventional method after that is mentioned. As a site-specific mutation introducing method, the approach (the GYAPPUDO duplex method, Nucleic Acids Res., 12, 9441-9456 (1984)) of using amber mutation, the approach by the PCR method using the primer for variation installation, etc. are mentioned here, for example.
About the number of the amino acid changed above, they are specifically 1, some, or more than it at least 1 residue. The number of these alterations should just be the range which can find out adiposity acceleration activity.
Moreover, the alteration which relates to especially the permutation of amino acid among said deletion, addition, or a permutation is desirable. The permutation concerned has a more desirable permutation to the amino acid which has the property which was [description / on hydrophobicity, a charge, pK, and a spacial configuration] similar. As such a permutation, a permutation within the group of an i glycine, alanine;ii valine, isoleucine, and leucine;iii aspartic acid, glutamic acid, an asparagine, a glutamine, iv serine, a threonine;v lysine, an arginine, a histidine;vi phenylalanine, and a thyrosin is mentioned, for example.
In this invention, "array identity" means the identity and homology of the array between two DNA or two protein. The above "array identity" is crossed to the field of the array for a comparison, and is determined by comparing two arrays which changed alignment into the optimal condition. Here, DNA or protein for a comparison may have addition or deletion (for example, gap etc.) in the optimal alignment of two arrays. About such array identity, Vector NTI is used and it is a ClustalW algorithm (it is computable by creating alignment using Nucleic Acid Res. and 22 (22):4673-4680 (1994).), for example. In addition, array identity is measured using array analysis software and the analysis tool specifically offered in Vector NTI, GENETYX-MAC, or a public database. Generally said public database is available in homepage address http://www.ddbj.nig.ac.jp.
Although the array identity in this invention should just be 80% or more, it is 95% or more more preferably 90% or more.
the hybridization which exists above (h) and which is related for "hybridizing under stringent conditions" and is used here -- for example, Sambrook J., Frisch E.F., Maniatis T. work, the 2nd edition (Molecular Cloning 2nd edition) of molecular cloning, and cold one Spring Harbor It can carry out according to the usual approach indicated by laboratory issuance (Cold Spring Harbor Laboratory press) etc. moreover, "the bottom of a stringent condition" -- for example, 6xSSC (it NaCl(s) 1.5M [ ] --) 0.15M Set the solution containing citric-acid 3 sodium to 10xSSC. After making a hybrid form at 45 degrees C in the solution which contains formamide 50%, Conditions (Molecular Biology, John Wiley & Sons, and N.Y. (1989), 6.3.1-6.3.6) which are washed at 50 degrees C by 2xSSC can be mentioned. The salt concentration in a washing step can be chosen from the conditions (high stringency conditions) to 50 degrees C for example, by 2xSSC by 50-degree C conditions (low stringency conditions) to 0.2xSSC. The temperature in a washing step can be chosen from a room temperature (low stringency conditions) to 65 degrees C (high stringency conditions). Moreover, both salt concentration and

temperature are also changeable.
As for the adiposity acceleration protein which is about 6000 or more molecular weight, it is desirable that it is the protein secreted out of a cell membrane. Moreover, as for the adiposity acceleration protein which is about 6000 or more molecular weight, it is desirable that it is the intraperitoneal adiposity acceleration protein which is about 6000 or more molecular weight.
[0008]
As a more detailed example of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight in this invention For example, it sets to the base sequence which carries out the code of the amino acid sequence shown by (I) array number 1, and the amino acid sequence shown by (II) array number 1. The base sequence which carries out the code of the partial amino acid sequence to which deletion of the 26 amino acid was carried out from the amino terminus In (namely, the base sequence which carries out the code of the amino acid sequence shown from the amino acid from the 27th in the amino acid sequence shown by the array number 1 to the 491st), and the amino acid sequence of the above (III) (II) The base sequence which carries out the code of the amino acid sequence by which the methionine was added to the amino terminus, (IV) The base sequence shown by the nucleotide from the 1st nucleotide in the base sequence shown by the array number 2 to the 1476th, (V) In the base sequence shown by the array number 2, the base sequence shown by the nucleotide from the 79th nucleotide in the base sequence shown by (VI) array number 2 to the 1476th, and the base sequence of the above (VII) (VI) The thing containing the gene which has base sequences, such as a base sequence by which ATG was added to the five prime end, is mentioned. In addition, what is necessary is just to acquire these genes according to the usual gene engineering-approach (for example, approach indicated by Sambrook J., Frisch E.F., Maniatis T. work, 2nd edition [of molecular cloning] (Molecular Cloning 2nd edition), and cold spring harbor laboratory issuance (Cold Spring Harbor Laboratory press) etc.).
The base sequence shown by the array number 2 is a base sequence of cDNA corresponding to mRNA which has the base sequence which carries out the code of the amino acid sequence shown by the array number 1, and includes the base sequence of an untranslation region in 5' end and 3' end. The genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight in this invention has the structure, i.e., intron-exon structure, where contained the base sequence of the array number 2, and this base sequence was usually divided by the intron.
As a more detailed example of intron-exon structure Between the base numbers 57 and 58 on the base sequence shown by the array number 2 (between exons 1 and 2), Between the base numbers 232 and 233 (between exons 2 and 3), between the base numbers 318 and 319 (between exons 3 and 4), The structure containing the intron is mentioned to the part corresponding to between the base numbers 506 and 507 (between exons 5 and 6) between the base numbers 447 and 448 (between exons 4 and 5).
[0009]

In this description, "adiposity acceleration activity" expresses the adiposity acceleration activity in a fat cell. The fat cell used here is an intraperitoneal fat cell of mammalian, and can be prepared by the following approaches. That is, from an animal, extraction and after carrying out beating, this is digested for intraperitoneal fat tissue with organization resolvability enzymes, such as collagenase, and cell suspension is prepared. The fraction which is rich in a precursor adipose cell is prepared by carrying out fractionation of the obtained cell suspension according to centrifugal separation etc. according to the approach indicated by Shillabeer G et al., International Journal of Obesity 20, and S77-S83 grade, and collecting precipitate. After cultivating this so that it may become KONFURENTO, the differetiation-inducing substance which has prostagladin J2 activity can be added, and it can be made to be able to specialize to a mature fat cell, and can use for measurement of adiposity acceleration activity. After adding the adiposity acceleration protein which is about 6000 or more molecular weight of the measuring object to this mature fat cell and cultivating for several days to it, the quantum of the triglyceride can be extracted and carried out by isopropanol, and adiposity acceleration activity can be measured by comparing with the amount of triglyceride under this protein nonexistence.
Specifically, it can measure by the approach indicated by the international disclosure pamphlet 02nd/the example 4 in No. 10772, or 5. That is, as protein which has adiposity acceleration activity, the protein of 110% or more of rates of adiposity acceleration is mentioned, and the protein of 120% or more of rates of adiposity acceleration is mentioned preferably.
[0010]
As a nonhuman animal in this invention, for example, nonhuman mammals (an example, a rabbit, a dog, a cat, a guinea pig, a hamster, a mouse, Latt, a sheep, a goat, Buta, a horse, a cow, ape, etc.) etc. are used, the mammalian of Rodentia (Rodentia), such as a mouse, Latt, and a guinea pig, is desirable, and a mouse and Latt are especially suitable.
If some nonhuman animals in this invention are the organizations or cells of the animal origin concerned, there will be especially no limit. For example, perimeter [testis] fat tissue, postabdomen film fat tissue, mesentery fat tissue, a subcutaneous adipose tissue, Some bodies of various kinds of fat tissues, such as brown adipose tissue, the organization of others further, such as the heart, lungs, the kidney, a gallbladder, liver, the pancreas, a spleen, intestines, a testis (testis), the ovary, a uterus, a placenta, muscles, a blood vessel, a brain, a core, the thyroid, a thymus gland, and a mammary gland, etc. are mentioned. Moreover, body fluid, such as blood of the animal origin concerned, lymph, or urine, is also contained at some nonhuman animals in this invention.
Furthermore, it is not concerned with the existence of differentiation and proliferation potential about the cultured cell (the cell which stock-ized the primary cell culture and this primary cell culture of the oneth generation which were extracted is included) obtained by isolating and cultivating the cell contained in the above-mentioned organization, an organ, or body fluid, an extract, each organ of a developmental stage [in / it does not come to accept it and / a fetus

germ] or the culture of the cell which carries out a paralysis, and an embryonic stem cell, either, but is contained at some nonhuman animals.

[0011]

The first mode of this invention is related with an individual, its descendant animal, or those some of knockout nonhuman animal.

this invention nonhuman animal is offered as the heterozygote currently destroyed so that one [all in the diplont of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight of a somatic cell chromosome, or] allyl compound may be permuted by the functional deletion mold mutant alle or one [all in the diplont of said genome gene or] allyl compound may carry out deletion of the function, or a homozygote.

[0012]

this invention nonhuman animal is the well-known target modifying-gene method (the gene targeting method: for example). Masami Muramatsu, Yamamoto elegant edit, the "3rd edition of experimental-medicine separate volume new revision gene engineering handbook revision" (in 1999) It applies to the usual approaches, such as the Yodosha issuance, 239 pages - 256 pages, Science 244:1288-1292, and 1989, correspondingly. The genome gene made into the object on the chromosome of ES (embryonic stem) cell of the nonhuman animal which performs the genome gene alteration of the adiposity acceleration protein which is about 6000 or more molecular weight One [all in the diplont of (the genome gene / namely, / of the adiposity acceleration protein concerned), or] allyl compound For example, the targeting vector (selective marker gene promotor loess recombination vector) which removed the promoterregion of a selective marker gene is used. After destroying by the homonous rearranging method (namely, the promotor trap method) which a part of object gene on a chromosome transposes to a targeting vector and making this mix in the blastocyst of a parent animal, it is effective to create the chimera primitive mouse which is ** to which made continue embryonic differentiation and it was made to give birth. By this approach, an embryonic stem cell etc. is used as a totipotency cell. As a totipotency cell, a mouse (Nature 292:154-156, 1981), Latt (Dev.Biol.163(1):288-292, 1994), The embryonic stem cell etc. is established about the ape (Proc.Natl.Acad.Sci.U.S.A.92 (17):7844-7848, 1995) and the rabbit (Mol.Reprod.Dev.45(4):439-443, 1996). Moreover, EG (embryonic germ) cell is established about Buta (Biol.Reprod 57(5):1089-1095, 1997). Therefore, although producing for these animal species is desirable as for this invention nonhuman animal, the mouse with which the technique is ready about especially production of a knockout animal is the optimal.

[0013]

The concrete procedure of production of this invention nonhuman animal is explained below. First, a variation DNA fragment which is destroyed so that the DNA fragment of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight is isolated, genetic manipulation of the DNA fragment is carried out within a test tube, the genome gene concerned may be permuted by the functional deletion mold mutant alle or said genome gene may carry out deletion of the function is produced. Isolation of DNA of the genome

gene concerned is acquired by screening nonhuman animal genomic DNA libraries, such as a mouse, using the oligonucleotide probe compounded based on the cDNA base sequence of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight of the well-known Homo sapiens origin etc. Moreover, DNA of the genome gene made into the object also by the PCR method which makes a primer the synthetic oligonucleotide equivalent to the part or ends of this cDNA can be obtained. In addition, also when aimed at nonhuman animals other than a mouse, DNA of the gene of the various nonhuman animal origins concerned can be isolated by the aforementioned approach of making the oligonucleotide compounded based on the aforementioned cDNA base sequence a probe or a primer. Furthermore, based on the base sequence of above cDNA, the structure of the genome gene concerned is analyzed in a detail, and the base sequence of the intron is solved in the intron-exon structure list in the genome gene concerned. If it changes by the permutation, deletion, and/or addition including the DNA fragment of the genome gene concerned based on the solved information so that it may differ from the base sequence of a part of [at least] DNA in the structural protein coding region of the gene concerned, a variation DNA fragment which is destroyed so that the genome gene concerned may be permuted by the functional deletion mold mutant alle or said genome gene may carry out deletion of the function is producible.

[0014]

Thus, the targeting vector for introducing variation into the genome gene of the adiposity acceleration protein with which totipotency cells, such as an embryonic stem cell, have some DNA fragments of the genome gene of the isolated adiposity acceleration protein which is about 6000 or more molecular weight using the variation DNA fragment changed and produced and which is about 6000 or more molecular weight is produced according to a well-known approach (for example, Science 244:1288-1292, 1989). A "targeting vector" is a DNA molecule for destroying the target gene by homonous recombination here, and in order to make easier selection of totipotency cells, such as an embryonic stem cell, the base sequence of a selective marker gene is usually included. For example, as an electropositive selective marker gene, a neomycin resistance gene, a beta-galactosidase gene, etc. can be raised, and the thymidine kinase gene of a herpes simplex virus, a diphtheria toxin A fragmentation gene, etc. can be raised as a shade sexual-selection marker gene. In addition, in case a targeting vector is built, the plasmid vector marketed as an object for targeting vector construction can also be used.

As an approach of producing the targeting vector preferably used in this invention for example, a part of [at least] DNA[in the structural protein coding region of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight -- in addition In being the protein with which the adiposity acceleration protein which is about 6000 or more molecular weight consists of an amino acid sequence shown by the array number 1 A part of [at least] DNA in the structural protein coding region of the genome gene concerned is the bases in an exon 3 and 4 fields (with "the base in an exon 3 and 4 fields" here). for

example, you may be all the bases in an exon 3 and 4 fields, and may be some bases in an exon 3 and 4 fields. it is -- things are desirable, it is more desirable that they are all the bases in exon 3 field and the base of the part in exon 4 field, and it is desirable that they are especially all the bases in an exon 3 and 4 fields. However, in exon selection here, when the number of bases of the field from the head of an exon 3 is set to 2+3n (n is the number of amino acid of the field from the head of an exon 3), since there is a possibility that the protein with which the translation frame following behind united the downstream region from the exon 2 in accordance with the down-stream gene of the structural protein coding region of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight may be produced, it is not desirable.] The gene cassette which has the selective marker gene by which promoterregion is removed concrete -- SA (splicing accepter) of the mouse EN2 origin -- IRES of the encephalomyocarditisecond Virus origin (internal ribosome entry site), pA of the SV40 origin in betageo with which it makes it come to unite beta-galactocidase and a neomycin resistance gene (polyadenylation signal) () [Mountford P., et al.,] [PNAS] 91 DNA which comes to process 4307 and 1994 flush end-ization is contained. :4303- And the selective marker gene promotor loess recombination vector which has a homologous base sequence is used for a part of DNA of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight. By permuting by DNA which is a part or all of the vector concerned, and contains said gene cassette Or by inserting in a part of [at least] DNA in the structural protein coding region of said genome gene DNA which is a part or all of the vector concerned, and contains said gene cassette The gene cassette which has the selective marker gene by which promoterregion is removed is contained. And the approach of producing a selective marker gene promotor loess recombination vector which has a base sequence [homologous / DNA / a part of / of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight] to the ends of said gene cassette can be raised. In this case, you may also build into the plasmid vector for targeting vector construction the DNA fragment which may carry out the permutation concerned or insertion, and is permuted or inserted and becomes as mentioned above to the plasmid vector for targeting vector construction into which the DNA fragment before being permuted or inserted as mentioned above was built beforehand.
Of course, said gene cassette may have a part of homologous base sequence to the base sequence of DNA of a genome gene to the selective marker gene order field by which promoterregion was removed.
"A homologous base sequence" is a base sequence which has identity or homology to the base sequence of DNA for a comparison here. Although it is not restricted especially if a trouble is not produced in case the homonous rearranging method is enforced For example, the base sequence which has 90% or more of array identity not continuously different three or more bases, the base sequence which has 95% or more of array identity to the base sequence of DNA for a comparison, and the base sequence which has 100% of array identity more preferably are raised preferably.

[0015]
The it is [containing the gene cassette which has the selective marker gene, for example, the resistance gene to cytotoxin, for example, the neomycin resistance gene, of G418 grade, by which promoterregion is removed in the above-mentioned targeting vector, and having a base sequence / homologous / DNA / a part of / of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight] important it, and other element will not be restricted, especially if a trouble is not produced in case the homonous rearranging method is enforced. In addition, this application may describe such a targeting vector a selective marker gene promotor loess recombination vector like the above-mentioned and the after-mentioned.
[0016]
Next, the above-mentioned targeting vector is introduced into totipotency cells, such as an embryonic stem cell of the nonhuman animal origins, such as a mouse, according to a well-known approach. Although a well-known electric pulse method, the liposome method, a calcium phosphate method, etc. can be raised as such a transgenics method, when the effectiveness of homonous recombination of an introductory gene is taken into consideration, the electric pulse method to totipotency cells, such as an embryonic stem cell, is desirable.
[0017]
Extract DNA of totipotency cells, such as an embryonic stem cell by which transgenics was carried out, and extracted DNA thus, by Southern blot analysis, PCR analysis, etc. The wild type genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight which exists on a chromosome (specifically a part of [at least] DNA in the structural protein coding region of the genome gene concerned), Homonous recombination takes place correctly between DNA which is a part of DNA of the above-mentioned targeting vector, and contains said gene cassette, and the cell from which the variation concerned moved is chosen as the wild type genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight on a chromosome. The change in the amount of gene expression of the adiposity acceleration protein which is about 6000 or more molecular weight can be investigated by carrying out an PCR real-time quantum (RT-PCR method: Absolute Quantification Real-Time Analysis) etc., and comparing the result about the selected embryonic stem cell and the selected wild type embryonic stem cell.
Incidentally, by the above approaches, only when the promotor of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight can be used and it is discovered within an embryonic stem cell after homonous recombination takes place to totipotency intracellular chromosomes, such as an embryonic stem cell, a selective marker gene is discovered and the property as selective markers, such as a resistance function to cytotoxin, is given. When aimed at the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight, it thinks for rising by leaps and bounds as compared with the case where it is aimed at other genome genes by the appearance effectiveness of a clone in which gene disruption of the homonous recombination is carried out

by happening in the target location by using the approach concerned.
[0018]
Thus, it pours in and mixes and, subsequently to the uterus of assumed parents, this chimera germ is transplanted to the blasting cyst (blastocyst) of wild type feminity nonhuman animals, such as a wild type feminity mouse which is in a pseudopregnancy condition according to the microinjection method and condensation method with the obtained variant genome gene of common use of totipotency cells, such as an embryonic stem cell. If embryonic differentiation is made to continue, when a nonhuman animal is a mouse, it will give birth about 21 days after as a candidate chimera nonhuman animal which is **, for example. After making a foster parent attach and breed the born candidate chimera nonhuman animal, the variant genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight sorts out the chimera nonhuman animal (F0) included in a genital system cell.
What is necessary is just to carry out sorting by observing the difference in the color of hair (hair color of the produced chimera nonhuman animal). (In this case, the acquisition effectiveness of a chimera nonhuman animal in which selection of a chimera nonhuman animal with the high rate that the variant genome gene was introduced and that rearrange and totipotency cells, such as an embryonic stem cell, occupy became easy, and the variant genome gene went into the genital system cell as a result can be raised.)
Other sortings may extract DNA from bodily [some] (for example, tail head), and may be carried out by Southern blot analysis, PCR analysis, etc.
About the descendant (F1) by whom the variant genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight is got by mating of the chimera nonhuman animal included in a genital system cell, and the wild type nonhuman animal which is this animal species By performing Southern blot analysis, PCR analysis, etc. which furthermore used the extract DNA from bodily [some] (for example, tail head) as ingredient The heterozygote (nonhuman animal which has the genome gene of the adiposity acceleration protein with which the allyl compound was destroyed in diplont, and which is about 6000 or more molecular weight) with which the variant genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight was introduced is identified.
Moreover, for example, perimeter [testis] fat tissue, postabdomen film fat tissue, mesentery fat tissue, To various kinds of fat tissues, such as a subcutaneous adipose tissue and brown adipose tissue, and a pan, the heart, lungs, The kidney, a gallbladder, liver, the pancreas, a spleen, intestines, a testis (testis), the ovary, a uterus, a placenta, By carrying out PCR real-time quantitative analysis or NOZAN blot analysis made from the RNA extract from other organization part or fetus germs, such as muscles, a blood vessel, a brain, a core, the thyroid, a thymus gland, and a mammary gland, and comparing the result The change in the amount of adiposity acceleration protein gene expression which are about 6000 or more molecular weight can be investigated. furthermore, ELAISA (Enzyme Linked Immunosorbent Assay) made from the parts (the ground thing, the ground intercept, etc.) or body fluid of said body (blood, urine, etc.) — the change in the

amount of adiposity acceleration protein which are about 6000 or more molecular weight can be investigated by enforcing law and comparing the result.
The heterozygote which holds the variant genome gene of the adiposity acceleration protein which is about 6000 or more created molecular weight holds the variant genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight stably in all the reproductive cells and somatic cells, and can transmit the variation to a descendant animal efficiently by mating etc. Therefore, the change in the amount of the protein which is heterozygote identification, the above-mentioned amount of gene expression, or the above-mentioned gene expression product concerned can be similarly investigated about the descendant animal after them F1.
in addition, in the destroyed gene influencing generating and differentiation of a fetus individual generally and not drawing viviparous fatality Although the genotype of a homozygote (nonhuman animal which has the genome gene of the adiposity acceleration protein with which all the allyl compounds in diplont were destroyed, and which is about 6000 or more molecular weight), heterozygote, and a wild type is created according to the probability in accordance with Mendel's laws in the individual after F1 On the other hand, when the gene which consists of a base sequence which carries out the code of the amino acid sequence of indispensable protein to generating and differentiation of the fetus of the used nonhuman animal is destroyed, survival and birth of heterozygote or a homozygote of a nonhuman animal become difficult, and serve as viviparous fatality. moreover, the characteristic with which this is predicted to be a actual natality rate when heterozygote is obtained -- it can guess by comparing the probability of an appearance.

[0019]

Thus, it sets to the produced nonhuman animal or its descendant animal (namely, the individual of a knockout nonhuman animal or its descendant animal). Diet load breeding which combined general diet, a high fat food, high sucrose foods, or these (for example, high fat high sucrose (HH) foods of this description example etc. are illustrated.) OGTT(carbohydrate tolerance test: see fluctuation of blood sugar level [after carrying out the load of the sugar of a constant rate], or insulin value)/after carrying out ITT (insulin loading test: see fluctuation of the blood sugar level after carrying out the load of the insulin of a constant rate, or an insulin value), various blood/urinalyses (analysis of the metabolic turnover parameter of sugar or a fat), and various pathology structure analysis (various fat tissues --) Weight change of liver, the kidney, the pancreas, muscles, a blood vessel, etc. and these organization intercepts are dyed. It carried out observing the amount of others and insulins, the amount of glycogen, etc., and the difference in the physiological function of the knockout mouse of this invention and a wild type mouse was investigated. [condition / of a fat cell, a liver cell, or the Langerhans beta cell] Incidentally, both OGTT and ITT are the typical trials used as the index at the time of being able to evaluate blood-sugar-regulation capacity (glucose tolerance) objective, and evaluating the abnormalities in an insulin (secretion and metabolic turnover), and a pancreas beta cell function.

Consequently, the knockout nonhuman animal of this invention showed fasting-blood-sugar lifting and glucose tolerance lowering compared with the wild type nonhuman animal as shown in this description example. moreover, a TORIGURISE resaler [in / by breeding with high-calorie-diet food / liver] -- the id -- the opinion on which an amount and the amount of blood cholesterol levels rise was shown. These opinions showed having the description that the knockout nonhuman animal of this invention presents abnormalities to the function of a glycolipid metabolic system.
From the above-mentioned result, the knockout nonhuman animal of this invention, the part and a wild type nonhuman animal, or its part is compared, and the difference in the onset condition of the disease (it is a concept including diseases, such as metabolic disorders, such as glucose tolerance lowering, diabetes mellitus, hyperlipidemia, hypertension, and arteriosclerosis, and a coronary artery disease, angina pectoris, myocardial infarction, cardiovascular disturbance, etc.) accompanied by a glycolipid metabolic turnover malfunction can be known.
Furthermore, symptoms analysis can also be performed also about the disease in which the adiposity acceleration protein which is about 6000 or more molecular weight participates by conducting the growth differentiation in the period of from the nonhuman animal of this invention, or a part of its fetus until fatal, development, observation of living activities, pathology histological inspection, or biochemical inspection.
[0020]
That is, the 2nd mode of this invention is related with the model animal of the disease (for example, diseases, such as diseases, such as metabolic disorders, such as glucose tolerance lowering, diabetes mellitus, hyperlipidemia, hypertension, and arteriosclerosis, and a coronary artery disease, angina pectoris, myocardial infarction, cardiovascular disturbance, are mentioned) accompanied by a glycolipid metabolic turnover malfunction which consists of a knockout nonhuman animal of above-mentioned this invention.
The amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight is falling intentionally compared with a wild type, and the knockout nonhuman animal of this invention can express the disease in which the protein concerned participates. That is, they are specifically the following opinions by the abnormalities of glycolipid metabolism as above-mentioned. :
(a) By breeding with standard foods or high-calorie-diet food, fasting blood sugar rises as compared with a wild type nonhuman animal.
(b) By breeding with standard foods or high-calorie-diet food, glucose tolerance falls as compared with a wild type nonhuman animal.
(c) By breeding with high-calorie-diet food, the amount of blood cholesterol levels rises as compared with a wild type nonhuman animal.
(d) By breeding with high-calorie-diet food, the amount of liver triglyceride rises as compared with a wild type nonhuman animal.
******.
Therefore, since symptoms analyses, such as a disease accompanied by 1

glycolipid metabolic turnover malfunction, are performed as a model animal of the disease accompanied by a glycolipid metabolic turnover malfunction, the knockout nonhuman animal of this invention can be used in order to evaluate glycolipid metabolic regulation capacity, such as two drugs or drugs candidate matter.
In this description, symptoms analysis means analyzing all change of the in the living body it produces in the living body according to the disease accompanied by a glycolipid metabolic turnover malfunction.
That is, the knockout nonhuman animal and wild type nonhuman animal of this invention which were bred in standard diet conditions or diet nature obesity induction conditions (diet load test) are compared, and it can carry out by conducting the growth differentiation in the period of from fetus until fatal, development, observation of living activities, pathology histological inspection, or biochemical inspection. They are others [Yamamura / Ken-ichi / "transgenic animal"] that what is necessary is just to follow the approach which this contractor usually uses about the observation and inspection in these symptoms analyses. Editing (KYORITSU SHUPPAN Co., Ltd.)" etc. can be referred to.
For example, the gene which is set in the organization concerned and by which the manifestation is guided or controlled can be identified by analyzing total RNA extracted from the tissue of the knockout nonhuman animal of this invention by approaches, such as PCR real-time quantitative analysis or NOZAN blotting analysis, and comparing with the value in a wild type nonhuman animal. From the result, it can search for the marker gene to which a manifestation is changed in the disease accompanied by a glycolipid metabolic turnover malfunction, as a result can also consider as the index of a causative agent break through of a disease.
For the knockout nonhuman animal of this invention, it actually turned out that the amount of manifestations of a glycolipid metabolic turnover related factor is changed as shown in this invention example. That is, it turns out that the manifestation of ACO in FAS [in PEPCK in liver and G6P, and a brown fat cell / UCP1 and FAS], and a white fat cell is going up, respectively, and the existence of a disease in the animal used in disease modeling accompanied by a glycolipid metabolic turnover malfunction can be judged by making these manifestations into an index.
On the other hand, how to evaluate glycolipid metabolic regulation capacity, such as drugs or drugs candidate matter, is explained below at a detail.
[0021]
The 3rd mode of this invention is related with the assessment approach of the glycolipid metabolic regulation capacity in an examined substance of using the knockout nonhuman animal of this invention, or its part.
The produced nonhuman animal, or its descendant animal or its part (namely, an individual, its descendant animal, or those some of knockout nonhuman animal) is useful in fields, such as drugs development to the disease relevant to a glycolipid metabolic turnover etc. Moreover, the nonhuman animal concerned, or its descendant animal or its part can be used in order to evaluate the glycolipid metabolic regulation matter.

[0022]
The assessment approach of this invention,
(1) The first process which contacts an examined substance to the knockout nonhuman animal of this invention, or its part,
(2) The second process [contrast / measure the index value which has a correlation in the amount of manifestations or the amount concerned of the adiposity acceleration protein which is about 6000 or more molecular weight in the nonhuman animal to which said examined substance was contacted, or its part, and],
It consists of the third process which evaluates the glycolipid metabolic regulation capacity of an examined substance based on the comparison result of (3) and (2).
[0023]
In this description, the glycolipid metabolic regulation matter expresses the matter which has glycolipid metabolic regulation capacity.
"Glycolipid metabolic regulation capacity" expresses the capacity (biosynthesis of fatty acid and/or fatty-acid metabolic regulation capacity) which controls the capacity (sugar metabolic regulation capacity) which controls a glycolysis, a glyconeogenesis, and sugar incorporation in muscles, liver, or fat tissue, lipid composition, or lipid decomposition. Furthermore, the capacity which controls the cholesterol composition metabolic turnover (cholesterol production controllability) which used said fatty acid as the raw material, production consumption of energy (ATP), and a living body's insulin susceptibility is also included in the concept of glycolipid metabolic regulation capacity.
[0024]
In this description, as an examined substance, there is especially no definition, it is a nucleic acid, a peptide, protein (the antibody to this invention protein is included), an organic compound, an inorganic compound, etc., and a cell extract, the manifestation product of a gene library, a synthetic low-molecular organic compound, a synthetic peptide, a synthetic nucleic acid, a natural compound, etc. are mentioned.
[0025]
In the above-mentioned assessment approach, when using the knockout nonhuman animal of this invention, the nonhuman animal concerned is bred by the breeding conditions which give the usual breeding conditions or the high-calorie-diet food by the standard diet. There is especially no definition about the food intake approach of a diet, a free food intake is carried out or the food intake of the constant rate is carried out to fixed time amount. What is necessary is just to use the diet currently suitably used widely by this contractor as a standard diet here according to each animal species.
In this description, although "high-calorie-diet food" expresses the high fat high sucrose (HH) foods which combined a high fat food, high sucrose foods, or these, it is controlling the conditions of the presentation and food intake, and it is possible to guide the onset of the glycolipid metabolic error disease of diet nature more effectively. The fat of a unit diet component is specifically chosen by 10-60kcal% (35kcal % of standard ****) width of face, and an animal lipid can be

further adjusted 0.5 to 10 times as compared with a vegetable lipid. Moreover, sucrose can be chosen from the carbohydrate component of a unit diet component by 0-35kcal% width of face.
In this description with "the time of high-calorie-diet intake (load breeding)" It is not what shows the event of the arbitration in the breeding period under the adjustment foods conditions of a fixed period, and limits a stage and a period, a count, and an amount in the period of from fetus until fatal. Moreover, change of symptoms is observable by carrying out the fast of about 2 to 24 hours temporarily, and carrying out biochemical inspection during a period. It breeds by dividing into the animal group from which diet conditions differ still more preferably, and after always carrying out comparison observation of each group with time about the parameter (weight, momentum, blood pressure, a food intake condition, biochemical inspection) in connection with the symptoms accompanying living activities or a glycolipid metabolic error, pathology histological inspection is conducted. As an item in biochemical inspection, although the blood sugar level, the amount of cholesterol, the amount of phospholipid, the amount of triglyceride, free fatty acid, an insulin value, a leptin value, the amount of manifestations of a glycolipid metabolic turnover related factor, etc. are illustrated, it is not limited to this.
It can mean medicating the nonhuman animal concerned with an examined substance in this description, saying "an examined substance is contacted to the knockout nonhuman animal of this invention, or its part", or contacting an examined substance to some of nonhuman animals concerned, and can carry out by the approach currently used widely by this contractor. What is necessary is for there to be especially no definition in the medication method, and just to medicate it with it taking-orally-wise or parenterally, when medicating the nonhuman animal concerned with an examined substance. As a parenteral medication method, intravenous administration, hypodermic administration, intracutaneous administration, intraperitoneal administration (ip), intrarectal administration, dermal administration (spreading), etc. can be mentioned.
There is especially no definition in the gestalt of an examined substance, and it can be used for it as a solid-state, a liquid, mixture with a basis, suspension, or a solution. When considering as suspension or a solution, water, pH buffer solution, a methyl cellulose solution, a physiological saline, an organic solvent water solution (as an organic solvent, ethanol and dimethyl sulfoxide are usually used.), etc. are used. It is used in order to mention oils, such as a glycerol and squalane, etc. as a basis and to mainly prepare the examined substance for spreading.
[0026]
What is necessary is to mention the approach of detecting and measuring the amount of RNA of this protein, and the approach of detecting and measuring this amount of protein as a measuring method of "the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight", and just to carry out like the measuring method of the amount of manifestations of the glycolipid metabolic turnover related factor mentioned later, or an approach given in an example.

As "the index value which has a correlation in the amount of manifestations of the adiposity acceleration protein which is about 6000 or more molecular weight" in the second process of said assessment approach,
(a) Usually, the blood sugar level at the hungry time in breeding or breeding with high-calorie-diet food,
(b) Usually, glucose tolerance in breeding or breeding with high-calorie-diet food,
(c) The amount of blood cholesterol levels at the time of breeding with high-calorie-diet food,
(d) the liver TORIGURISE resaler at the time of breeding with high-calorie-diet food -- the id -- an amount,
(e) The amount of manifestations of a glycolipid metabolic turnover related factor ******** -- things are made.
The maximum reaction acceleration approach (Glucoroder-NXA&T, Inc. manufacture) by the GOD (Glucose oxidase) immobilized enzyme electrode well known to this contractor as a measuring method of the blood sugar level, an approach given in this description example, etc. can be used.
As a measuring method of glucose tolerance, OGTT (carbohydrate tolerance test) or ITT (insulin loading test) is mentioned, preferably, an area under the curve value (AUC:area under the curve) can be computed from these results, it can consider as the index of glucose tolerance lowering with the increment, and the approach specifically indicated by this description example can be used.
The approach by the enzyme-immune absorbance analysis method kit (Roche Dianostics) well known to this contractor as a measuring method of the amount of blood cholesterol levels etc. can be used.
or a liver TORIGURISE resaler -- the id -- the approach which was well learned to this contractor as a measuring method of an amount and which was indicated by the acetylacetone method (Fletcher, M.J.:Clin.Chim.Acta, 22,339-397 (1968) and Sardesai, V.M.:Clin.Chim.Acta, 14,156-161 (1968)), an approach given in this description example, etc. can be used.
In addition, they are the approach well learned to this contractor about the biochemical analysis technique of the knockout nonhuman animal of this invention, "The Journal of Clinical Investigation, April2002, vol.109, no.8" (Robert V.Farese Jr. work) or "Nature Genetics, vol.25, and may 2000 (the approach indicated by Robert V.Farese Jr. work" etc. is mentioned.).
[0027]
The enzyme on this description and in connection with a glycolysis, a glyconeogenesis, and sugar incorporation with a glycolipid metabolic turnover related factor, The factor in connection with control of the metabolic turnover of a lipid, or a biosynthesis, and the cholesterol composition metabolic turnover which made this the raw material, It is the factor which changes also to the control about production consumption of energy, and the factor which participates in a living body's insulin susceptibility further, and 1 or two or more factors which are specifically chosen from PEPCK, G6P, UCP1, FAS, ACO, etc. are mentioned. When measuring the amount of manifestations of this glycolipid metabolic turnover related factor, the approach using RNA as the measuring object and the approach

using protein as the measuring object are mentioned. Each is stated to a detail below.

1) When RNA is used as the measuring object

When using RNA as a biological material, the amount of manifestations of this glycolipid metabolic turnover related factor is carried out by detecting and measuring the PEPCK gene, G6P gene, UCP1 gene, the FAS gene, or ACO gene expression level per [which is contained in a biological material] total RNA unit quantity.

Although especially definition does not have the origin animal species of a glycolipid metabolic turnover related factor gene, the gene of the same kind origin as this invention nonhuman animal is usually used.

[0028]

In this description, especially a PEPCK gene is used with the PEPCK gene (Genbank Accession No.AF009605 or array number 26) of the mouse origin, and the meaning which includes the homologue, unless reference is made. As a homologue, an isoform, an orthochromatic log [for example, the PEPCK gene (Genbank Accession No.A.I. Artificial Intelligence635748, BC023978, BM768055) of the Homo sapiens origin], etc. are mentioned.

In this description, especially G6P gene is used with G6P gene (Genbank Accession No.BC013448 or array number 28) of the mouse origin, and the meaning which includes the homologue, unless reference is made. As a homologue, an isoform, G6P gene (Genbank Accession No.U01120) of orthochromatic log [, for example, the Homo sapiens origin, etc. are mentioned.

In this description, especially UCP1 gene is used with UCP1 gene (Genbank Accession No.BC012701 or array number 30) of the mouse origin, and the meaning which includes the homologue, unless reference is made. As a homologue, an isoform, UCP1 gene (Genbank Accession No.U28480, X51952) of orthochromatic log [, for example, the Homo sapiens origin, etc. are mentioned.

In this description, unless especially an FAS gene makes reference, the FAS gene (Genbank Accession No.AF127033 or array number 32) of the mouse origin, the FAS gene (Genbank Accession No.U29344, U52428) of the Homo sapiens origin, etc. are mentioned.

In this description, unless especially an ACO gene makes reference, the ACO gene (Genbank Accession No.AF006688 or array number 34) of the mouse origin, the ACO gene (Genbank Accession No. AH000843, BC008767) of the Homo sapiens origin, etc. are mentioned.

[0029]

In a nonhuman animal, its descendant, or those part of this invention, the existence of the gene expression concerned or its extent can be measured by detecting the existence of a manifestation of the above-mentioned glycolipid metabolic turnover related factor gene (or gene product), or its extent. Namely, by carrying out PCR real-time quantitative analysis or NOZAN blotting analysis made from RNA extracted from the various organizations after carrying out diet load breeding By investigating the trend of other amounts of gene expression interlocked with

increase and decrease or this of the amount of glycolipid metabolic turnover related factor gene expression, the onset condition of the disease accompanied by a glycolipid metabolic turnover malfunction can be known, as a result it can also consider as the index of a causative agent break through of a disease.
That is, the biological material of the nonhuman animal origin of this invention, the primer of the glycolipid metabolic turnover related factor gene origin, or a probe can be contacted, and the amount of RNA combined with this primer or a probe can be measured by well-known approaches, such as a Northern blot technique, RT-PCR method, a DNA chip analysis method, and an in situ hybridization analysis method. The polynucleotide which has at least 15 bases which continue in the base sequence of a glycolipid metabolic turnover related factor gene as this primer or a probe, and/or its complementary polynucleotide are mentioned. the case where it uses as a primer -- usually -- 15bp-100bp -- desirable -- 15bp(s)- what has the base length of 15bp-35bp more preferably can be illustrated 50 bp. moreover -- the case where it uses as a detection probe -- usually -- the number of bases of a 15bp(s) - all array -- desirable -- 15bp(s)- what has the base length of 100bp(s) - 1kb more preferably can be illustrated 1 kb.
[0030]
When using a Northern blot technique, specifically The indicator of said probe is carried out with radioisotope (32P, 33P, etc.: RI), a fluorescent material, etc. After making it hybridize with RNA of the body tissue origin of the test subject who transferred it to the nylon membrane etc. according to the conventional method, The duplex chain of the primer (DNA or RNA) of the formed glycolipid metabolic turnover related factor origin, and all RNA of the biological material origin The approach of detecting the signal originating in the indicator object (RI or fluorescent material) of said primer with a radiation detector (BAS-1800II, the Fuji film company make) or a fluorescence detector, and measuring can be illustrated. Moreover, after carrying out the indicator of the probe DNA according to this protocol and making it hybridize with RNA of the biological material origin using AlkPhos Direct Labelling and Detection System (product made from Amersham PharamciaBiotech), the approach of detecting the signal originating in the indicator object of a probe with the multi-biotechnology imager STORM860 (product made from Amersham Pharmacia Biotech), and measuring can also be used.
[0031]
When using RT-PCR method, RNA and said primer of the biological material origin are made to hybridize, the PCR method can be performed according to a conventional method, and the approach of detecting the obtained magnification double stranded DNA can be illustrated. In addition, the approach of detection of the amplified double stranded DNA making transfer the approach of detecting the indicator double stranded DNA produced by performing Above PCR using the primer which carried out the indicator with RI or a fluorescent material beforehand, and the produced double stranded DNA to a nylon membrane etc. according to a conventional method, making hybridize it with this as a probe using the disease marker which carried out the indicator, and detecting etc. can be used. In addition, the generated indicator double-stranded-DNA product can be measured with a

horse mackerel RENTO 2100 biotechnology analyzer (YOKOGAWA analytical systems company make) etc. Moreover, according to this protocol, RT-PCR reaction mixture can be prepared by SYBR Green RT-PCR Reagents (Applied Biosystems shrine make), it can be made to be able to react by ABI PRIME 7700 Sequence Detection System (Applied Biosystems shrine make), and this reactant can also be detected.

[0032]

Moreover, the DNA chip which stuck said primer or probe of this invention as a DNA probe (a single strand or 2 chains) when DNA chip analysis was used can be prepared, and the approach of making hybridize with cRNA prepared by this with the conventional method from RNA of the body tissue origin, making combine the double strand of DNA and cRNA which were formed with the indicator probe prepared from said primer or probe of this invention, and detecting can be mentioned. Moreover, the DNA chip in which detection of the gene expression level of a PEPCK gene, G6P gene, UCP1 gene, an ACO gene, or an FAS gene and measurement are possible can also be used as the above-mentioned DNA chip.

[0033]

2) When protein is used as the measuring object

When using the solution which contains protein as a biological material, it carries out by detecting and measuring the amount of glycolipid metabolic turnover related factors which can be combined with this antibody by making the amount of glycolipid metabolic turnover related factors contained in a biological material react with the antibody which can recognize this glycolipid metabolic turnover related factor.

Although especially definition does not have the origin animal species of an antibody which can recognize a glycolipid metabolic turnover related factor, the antibody of the same kind origin as this invention nonhuman animal is usually used.

PEPCK, G6P, UCP1, and FAS or ACO** is specifically mentioned as a glycolipid metabolic turnover related factor, and the protein in which a code is carried out by the above-mentioned glycolipid metabolic turnover related factor gene as the amino acid sequence, respectively can be mentioned.

[0034]

Especially a limit does not have the antibody of said glycolipid metabolic turnover related factor in the gestalt, it may be the polyclonal antibody which makes said glycolipid related factor the immunogen, or you may be the monoclonal antibody, and the antibody which has antigen affinity to the polypeptide which continues at least among the amino acid sequences which constitute the glycolipid related factor concerned further, and which consists of 15 amino-acid extent preferably is usually contained in the antibody of this invention eight amino acid three amino acid.

[0035]

The manufacture approach of these antibodies is already common knowledge, and can also manufacture the antibody of this invention according to these conventional methods (Current protocols in Molecular Biology edit.Ausubel et

al.(1987) Publish.John Wiley and Sons.Section 11.12-11.13). It is possible to compound the oligopeptide which has the partial amino acid sequence of one of this invention protein concerned according to a conventional method according to a conventional method with Escherichia coli etc., using PEPCK, G6P, UCP1, and FAS or ACO discovered and refined, to carry out immunity to nonhuman animals, such as a rabbit, and to specifically obtain from the blood serum of this immune animal according to a conventional method, when the antibody of this invention is a polyclonal antibody. PEPCK which was discovered and refined with Escherichia coli etc. on the other hand according to the conventional method in the case of the monoclonal antibody, Immunity of the oligopeptide which has G6P, UCP1, FAS or ACO, or the partial amino acid sequence of these protein is carried out to nonhuman animals, such as a mouse. The spleen cell and myeloma cell which were obtained It can obtain out of the hybridoma cell which was made to carry out cell fusion and was prepared (). [Current protocols in Molecular Biology edit.Ausubel et al.(1987) Publish.] John Wiley and Sons.Section 11.4-11.11.

[0036]

Moreover, the protein used for production of an antibody can be obtained based on the array information on the gene of the glycolipid metabolic turnover related factor offered by this invention (the array numbers 27, 29, 31, and 33 or 35) by culture of DNA cloning, construction of each plasmid, the transfection to a host, and a transformant, and actuation of recovery of the protein from a culture. These actuation can be given to this contractor according to a known approach or an approach (Molecular Cloning, T.Maniatis et al., CSH Laboratory (1983), DNA Cloning, DM.Glover, and IRL PRESS) (1985) given in reference. It can carry out by specifically collecting object protein from the culture which creates the recombinant DNA (expression vector) which the gene which carries out the code of the glycolipid metabolic turnover related factor can discover in a desired host cell, introduces and carries out the transformation of this to a host cell, cultivates this transformant, and is obtained. Moreover, these glycolipids metabolic turnover related factor can also be manufactured by the general chemosynthesis method (peptide synthesis) according to the information on the amino acid sequence offered by this invention (the array numbers 27, 29, 31, and 33 or 35). Specifically, the liquid phase synthesis method and solid phase synthesis method which were indicated by "the foundation of peptide synthesis and the experiment" (the Izumi store Nobuo work, Maruzen, 1987 issuance) can be used.

[0037]

In addition, not only the protein shown in the array numbers 27, 29, 31, and 33 or 35 but its homologous object is included by the glycolipid metabolic turnover related factor of this invention. The protein which 1 or two or more amino acid consist of deletion and an amino acid sequence permuted or added, has biological functions equivalent to the function of the protein shown by the above-mentioned array number, or has equivalent activity in immunological activity as this homologous object in the amino acid sequence shown by the above-mentioned array number can be mentioned. In immunological activity, an antibody [as opposed to / being equivalent / a glycolipid metabolic turnover related factor] and

the protein which has combining ability specifically can be mentioned here.

[0038]

"contrast" in the second process of this invention assessment approach -- for example

(a) the case where the first process and the second process, and the same process are carried out for this invention nonhuman animal used by the assessment approach concerned, and the wild type nonhuman animal which is this animal species -- or

(b) Mean the case where used the quality of contrast (positive control, negative control) instead of the examined substance, and the first process and the second process, and the same process are carried out etc.

[0039]

If the glycolipid metabolic regulation capacity in the nonhuman animal of this invention is the glycolipid metabolic regulation capacity of the examined substance in a wild type nonhuman animal, and more than equivalent in the above (a), it can be estimated that the examined substance concerned has glycolipid metabolic regulation capacity. On the other hand, if the glycolipid metabolic regulation capacity in the nonhuman animal of this invention is smaller than the glycolipid metabolic regulation capacity of the examined substance in a wild type nonhuman animal, it can be estimated that the examined substance concerned does not have the glycolipid metabolic regulation capacity resulting from the adiposity acceleration protein which is about 6000 or more molecular weight.

In the above (b), positive control or negative control is mentioned as quality of contrast. Positive control expresses the matter of the arbitration which has glycolipid metabolic regulation capacity, and an insulin etc. is specifically illustrated. Moreover, as negative control, the solvent contained in an examined substance, the trial system solution used as a background, etc. are raised.

If the glycolipid metabolic regulation capacity of an examined substance is larger than the glycolipid metabolic regulation capacity of the quality of contrast when considering quality of contrast as negative control, it can be estimated that the examined substance concerned has glycolipid metabolic regulation capacity. on the other hand, the glycolipid metabolic regulation capacity of an examined substance is comparable as the glycolipid metabolic regulation capacity of the quality of contrast -- or if small, it can be estimated that the examined substance concerned does not have glycolipid metabolic regulation capacity.

Moreover, when considering quality of contrast as positive control, extent of the glycolipid metabolic regulation capacity of an examined substance can be evaluated by measuring the glycolipid metabolic regulation capacity of an examined substance, and the glycolipid metabolic regulation capacity of the quality of contrast.

[0040]

Since it has glycolipid metabolic regulation capacity, the matter (glycolipid metabolic regulation matter) chosen based on the glycolipid metabolic regulation capacity of the examined substance obtained by this invention assessment approach can be used as drugs which have effectiveness in the therapy and

prevention to the disease (glucose tolerance lowering, diabetes mellitus, hyperlipidemia, hypertension, arteriosclerosis, a coronary artery disease, angina pectoris, myocardial infarction, or cardiovascular disturbance) accompanied by a glycolipid metabolic turnover malfunction etc. Furthermore, the compound guided from the matter obtained by this invention screening approach can be used similarly.

The matter obtained by this invention screening approach has the desirable acid addition salt which the salt may be formed, and a salt with an acid (the example, the inorganic acid, organic acid) or a salt (the example, alkali metal) permitted physiologically is used as a salt of the matter concerned, and is especially permitted physiologically. As such a salt, a salt with an inorganic acid (for example, a hydrochloric acid, a phosphoric acid, a hydrobromic acid, a sulfuric acid) or a salt with an organic acid (for example, an acetic acid, a formic acid, a propionic acid, a fumaric acid, a maleic acid, a succinic acid, a tartaric acid, a citric acid, a malic acid, oxalic acid, a benzoic acid, methansulfonic acid, benzenesulfonic acid) is used, for example. The matter obtained by this invention screening approach can be parenterally used in the form of injections, such as water, an axenic solution with the other liquid which can be permitted pharmacologically, or a suspension agent, in taking orally as the tablet and capsule which gave glycocalyx if needed, elixirs, a microcapsule agent, etc. For example, it can manufacture by mixing with the matter concerned with the unit dosage gestalt required of the pharmaceutical preparation implementation generally accepted with the support and the flavor agent which can be accepted physiologically, an excipient, a vehicle, antiseptics, the stabilizer, the binder, etc. Capacity with the directed range suitable for the amount of active principles in these pharmaceutical preparation is obtained. As an additive which can mix with a tablet, a capsule, etc., a flavor agent like plumping agents, such as gelatin, corn starch, tragacanth, a binder like gum arabic, an excipient like a crystalline cellulose, corn starch, gelatin, and an alginic acid, lubricant like magnesium stearate, cane sugar, a lactose or a sweetening agent like saccharin, peppermint, a dirt mono-oil, or a cherry etc. is used, for example. When dispensing unit form voice is a capsule, liquefied support still like fats and oils can be contained into said type of ingredient. The sterile constituent for injection can prescribe natural appearance vegetable oil, such as an active substance in a vehicle like water for injection, sesame oil, and coconut oil, etc. according to the usual pharmaceutical preparation implementation of making it dissolve or suspend etc. As aquosity liquid for injection, the isotonic solutions (for example, D-sorbitol, D-mannitol, a sodium chloride, etc.) containing the adjuvant of a physiological saline, grape sugar, or others etc. are used, for example, and you may use together with a suitable solubilizing agent (an example, ethanol), for example, alcohol, polyalcohol (an example, propylene glycol, polyethylene glycol), a nonionic surfactant (an example, polysorbate 80 TM, HCO-50), etc. As oily liquid, sesame oil, soybean oil, etc. are used and you may use together with benzyl benzoate, benzyl alcohol, etc. which are a solubilizing agent, for example. Moreover, a buffer (for example, a phosphate buffer, the sodium acetate buffer solution), aponia-ized agents (for example, a benzalkonium chloride, procaine hydrochloride, etc.),

stabilizers (for example, a human serum albumin, a polyethylene glycol, etc.), preservatives (for example, benzyl alcohol, a phenol, etc.), an antioxidant, etc. may be blended with the above-mentioned therapy and preventive. Suitable ampul is usually filled up with remedy constituents, such as a prepared parenteral solution.
[0041]
Thus, the pharmaceutical preparation obtained is safe, and since it is low toxicity, a medicine can be prescribed for the patient to Homo sapiens or mammalians (for example, Latt, a rabbit, a sheep, Buta, a cow, a cat, a dog, an ape, etc.), for example. Although it is different with the administration root an object disease and for administration etc., generally the dose of the matter concerned prescribes more preferably about 0.1-100mg of about 1-50mg of about 1-20mg of these these matter for the patient per day in an adult, for example, when administering the compound concerned orally (as weight of 60kg). It is convenient to take lessons from a day, when usually medicating an adult (as 60kg) with the matter concerned in the form of injections, for example, although the 1-time dose of the matter concerned changes with object diseases for administration etc. in prescribing a medicine for the patient parenterally, and to prescribe more preferably about about 0.01-30mg about about 0.1-20mg about about 0.1-10mg the matter concerned for the patient by the intravenous injection. The amount which converted into per 60kg also in other nonhuman animals can be prescribed for the patient.
[0042]
Although an example explains this invention below, this invention is not limited to these.
[Example 1]
[0043]
(Isolation and structural analysis of this gene)
The base sequence which carries out the code of the amino acid sequence of the adiposity acceleration protein which is about 6000 or more molecular weight of the target mouse origin in this example is indicated by GenBank AF234625 (mRNA, 2664bp) etc. The chromosome gene library of the mouse origin in order to acquire the clone containing the DNA fragment of the genome gene concerned (screening by the PCR method for CITB Mouse BAC(bacterial artifical chromosome Library) Cell line DJ7(129SV) DNA pools (Research Genetics shrine make) was carried out.) In this case, the used PCR method is standard Taq polymerase which uses the primer of the couple of primer GE-U7 (array number 3:GGC CAC AAA TTC CAG AGA ACA G) and GE-L7 (array number 4:CCA AAT GAG CAG ATG CCC CTA T). It was the reacting (Toyobo, Japan) method. in addition, the program of the PCR concerned -- a DNA danaturation (for 94 degrees C and 1 minute) -- continuing -- denaturation (for 94-degree-C and 1 minute)-annealing (for 55-degree-C and 30 seconds)-expanding (for 72 degrees C and 2 minutes) of 40 cycle -- it was the last expanding (for 72 degrees C and 1 minute) further. Consequently, one electropositive clone which amplifies the DNA fragment (850bp) which has the base sequence which is equivalent to the field from an exon 5 to an exon 6 among the DNA fragments of the genome gene of the adiposity

acceleration protein which is about 6000 or more molecular weight of the mouse origin was obtained.
[Example 2]
[0044]
(Construction of a targeting vector)
pCR-085 were prepared by [which have a base sequence equivalent to the field from an exon 2 to an exon 6] carrying out DNA fragment (about 9 kbp(s)) isolation, and carrying out cloning of this to vector pCR-XL (product made from Invitrogen) among the DNA fragments of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight of the mouse origin in the clone (BAC clone) obtained in the example 1. In this case, the used PCR method is Expand Long Template PCR system which uses the primer of the couple of primer U8ClaI-2 (array number 5:ATC GAT TCC TAC TTT GAA TGC CGT GAA) and primer L15SalI (array number 6:GCT GTC GAC TGG AAC AGA ATA GCC TGG AA) and which used baffer1. It was the reacting (Loche) method. in addition, the program of the PCR concerned -- a DNA danaturation (for 94 degrees C and 10 seconds) -- continuing -- denaturation (for 94-degree-C and 5 seconds)-annealing (for 60-degree-C and 5 seconds)-expanding (for 68 degrees C and 10 minutes) of 36 cycle -- it was the last expanding (for 68 degrees C and 20 minutes) further.
next, a pGT1.8IresBgeo vector (Grossler, A., Joyner, A.L., Rossant, J., Skarnes, wc:Science, and 244,443-465 (1989) --) Peter S.Nountford and Austin G.Smith:TIG the SA-IRIS-beta geo-pA gene cassette (SA (splicing accepter) of the mouse EN2 origin --) cut down by SalI from the well-known vector indicated by May 1995 and Vol.11 No.5 grade IRES of the encephalomyocarditisecond Virus origin (internal ribosome entry site), DNA which comes to process pA (polyadenylation signal) of the SV40 origin flush end-ization is used for betageo with which it makes it come to unite beta-galactocidase and a neomycin resistance gene. The inside of the DNA fragment of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight of the mouse origin started by pCR-085 to MunI, The targeting vector was built by replacing the DNA fragment which has a base sequence equivalent to the field from an exon 3 to an exon 4. Thus, homonous recombination of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight in an embryonic stem cell is carried out using the built targeting vector. Exon 2 which said targeting vector has by such homonous recombination on an original promotor's lower stream of a river which the genome gene concerned originally has - It permutes by DNA which consists of said SA-IRIS-beta geo-pA gene cassette-exon 6, and the structural protein coding region which is equivalent to the field to an exon 3 to a part of exon 4 as a result carries out deletion. Moreover, simultaneously, a frame shift arises and, henceforth [the exon 4 following the lower stream of a river of the gene cassette concerned], the translation product of the genome gene concerned is not produced accidentally as a result. By the gene cassette concerned by which the genome gene concerned was inserted in the lower stream of a river of the original promotor who originally has further again Since the promoterregion of the selective

marker gene which the gene cassette concerned has is removed The manifestation control in said genome gene of cell original and the same manifestation control will be received, and it sets to a knockout nonhuman animal or its part. For example, the resistance capacity over the cytotoxin of G418 grade given by beta-geo and coloring by X-Gal dyeing can investigate the manifestation from the genome gene concerned easily.

[Example 3]

[0045]

<Installation to the embryonic stem cell of a targeting vector, and homonous recombination>

The targeting vector built in the example 2 is cut by XhoI. Law is used. since it straight-line-izes -- electroporation (0.25kV, 0.5micro-Fx1000) -- RF8 embryonic stem cell which carried out trypsinization () [Meiner] VL et al. and Proc Natl 129 Sv/Jae mouse origin cell:Robertson indicated by Acad Sci USA.93:14041 and 1996 grades, E.J., and Teratocarcinomas and Embryonic Stem Cell: It preparation-possible-passed by the approach indicated by A Practical Approach (1987) etc., and introduced.

RF8 into which the targeting vector was introduced An embryonic stem cell The SNL cell which is a sustentacular cell cultivated by the bottom whole surface of a petri dish () [which included the expression vector of a neomycin resistance gene and the leukemia inhibitor (LIF) in fibroblast (STO cell)] [thing:McMahon AP and Bradley] After cultivating 37 degrees C on A, Cell 62:1073, and 1990 for 24 hours, it cultivated for 37 degrees C and seven - 15 days further in the neomycin content culture medium (G418:250microg [/ml] content). The clone of G418 resistance in which the colony was formed was selected after culture. Thus, after making the selected clone (embryonic stem cell) into the embryonic stem cell to which the homonous recombination on the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight took place and carrying out amplification culture of this, about a part of obtained embryonic stem cell, the DNA analysis by the PCR method about said genome gene was presented, and frozen preservation (-80 degrees C) was presented about other parts. In this case, the used above-mentioned DNA analysis was a method of reacting Expand Long Template PCR system using the primer of the couple of forward sense primer SU-screening3 (array number 7:AAC AAT ACC CAC CCA ACA CAA GCA) located in an exon 2, and reverse sense primer SA-screening AS2 (array number 8:CGC ACG CCA TAC AGT CCT CTT CAC) located in SA in betageo gene cassette. in addition, the program of the PCR concerned -- a DNA danaturation (for 94 degrees C and 5 seconds) -- continuing -- denaturation (for 94-degree-C and 2 seconds)-annealing (for 60-degree-C and 2 seconds)-expanding (for 68 degrees C and 2 minutes) of 36 cycle -- it was the last expanding (for 68 degrees C and 3 minutes) further.

Thus, it checked that homonous recombination had taken place to accuracy in the object location of the above-mentioned embryonic stem cell (that is, there needs to be magnification of the DNA fragment of about 1.3 kbp(s)).

in addition, when the POJINEGA method which made the general genome gene the

target is adopted Among totipotency cells, such as an embryonic stem cell by which positive selection was made, although totipotency cells, such as an embryonic stem cell by which homonous recombination is checked at about 10 - 50% of a rate, exist, usually Although about 1200 embryonic stem cell was screened when the genome gene of the adiposity acceleration protein which is about 6000 or more target molecular weight was made into a target in this example, the embryonic stem cell by which homonous recombination of the genome gene concerned was carried out was not obtained. On the other hand, when the above promotor trap methods were adopted, about ten percent of the selected clone was the homonous recombination embryonic stem cell made into the object.

[Example 4]

[0046]

(Southern-blotting analysis of the genome gene which a homonous recombination embryonic stem cell has)

Restriction enzyme digestion of the chromosome DNA of the homonous recombination embryonic stem cell obtained in the example 3 was carried out by EcoRV. Agarose gel electrophoresis was presented with the chromosome DNA fragment by which restriction enzyme digestion was carried out 0.8%, and the separated chromosome DNA fragment was imprinted using the turbo blotter (turbo blotter system made from Schneider&Shunel) to the nylon membrane (yes the Amersham make, bond N).

In order to acquire the probe originating in the intron 6 (namely, intron located between an exon 6 and an exon 7) of the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight Amplify the DNA fragment which has a base sequence equivalent to the field of until it is from an exon 6 to an exon 8. Forward sense primer GE-U12 (array number 9:GGG CAT CTG CTC ATT TGG TTA) located in an exon 6, Reverse sense primer GE-L12 (array number 10:CTC TTG TAA AGT ATT GAT ATC CAC G) located in an exon 8 is used. And said BAC clone is used as mold and it is Expand Long Template PCR system. It reacted. in addition, the program of the PCR concerned -- a DNA danaturation (for 94 degrees C and 5 seconds) -- continuing -- denaturation (for 94-degree-C and 2 seconds)-annealing (for 60-degree-C and 2 seconds)-expanding (for 68 degrees C and 4 minutes) of 36 cycle -- it was the last expanding (for 68 degrees C and 5 minutes) further.

After carrying out cloning of the amplified DNA fragment (3.5kbp) to pCR2.1 (Invitrogen), the DNA fragment (1.6kbp) was cut down by EcoRV from the plasmid concerned, and gel purification was presented with this.

Thus, Southern-blotting analysis of the chromosome DNA fragment (namely, DNA fragment of the genome gene origin which a homonous recombination embryonic stem cell has) with which the above was separated was carried out, using the obtained DNA fragment as a probe. In addition, in the case of a homonous recombination embryonic stem cell, the DNA fragment of 10kbp(s) can be detected, and, in the case of an un-homonous recombination embryonic stem cell (wild type embryonic stem cell), the DNA fragment of 16kbp(s) can be detected.

The result was shown in drawing 2 . Two clones which can check the homonous recombination in the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight of the target mouse origin in this example were found out so that clearly from drawing.

[Example 5]

[0047]

(Production of a heterozygosity knockout nonhuman animal (mouse))

Thus, the checked homonous recombination embryonic stem cell (namely, embryonic stem cell with a variant genome gene) was poured into the blasting cyst (blastocyst) of wild type feminity mouse 57BL/6J (made in Japanese Clare) which are in a pseudopregnancy condition according to the approach indicated by A.L.Joyner, "Gene Targeting-A Practical Approach" 2nd edition, Oxford UNIV, etc., and, subsequently to the uterus of assumed parents, this chimera germ was transplanted. When embryonic differentiation was made to continue, the candidate chimeric mouse gave birth about 21 days after. After making a foster parent attach and breed the born candidate chimeric mouse, in this example, the variant genome gene of the adiposity acceleration protein which is about 6000 or more target molecular weight sorted out the chimeric mouse (F0) included in a genital system cell. Sorting used the approach of observing the difference in the color of hair, and chose four individuals from the color of hair of an agouti by using the chimeric mouse of 90% or more of chimera **** as F0 mouse.

The selected chimeric mouse and the wild type mouse which is this animal species (namely, C57BL/6J mouse) were crossed, and heterozygote F1 mouse (mouse which has genome gene of adiposity acceleration protein with which allyl compound was destroyed in diplont, and which is about 6000 or more molecular weight) 38 individual into which the variant genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight was introduced was created. Furthermore, heterozygote F2 mouse was produced by crossing these F1 mice. About some heterozygote F1 mice, it applied as the heterozygote F1 mouse concerned and the wild type mouse which is this animal species (namely, C57BL/6J mouse), and the back cross was carried out.

[Example 6]

[0048]

(Genotype analysis of a knockout nonhuman animal (mouse))

The extract DNA from a mouse tail head is used as mold at the genotype analysis of a chimeric mouse. And forward sense primer Sumi-S6 (array number 11:TAA TGG TGG GTT GTT TGT TTG TCA AGG C) common to the wild type and homonous recombinant which are located in three kinds of following primer [(1) introns 2, (2) Reverse sense primer Sumi-AS2 (array number 12:GCA GTA CTC TAA AGT AGG CAA CCA ATG T) which carries out the complementation only to the intron 2 of a wild type, and reverse sense primer betageo-screening AS2 (array number 13:GAC CTT GCA TTC CTT TGG CGA GAG) which is located in IRIS in a (3) betageo gene cassette, and carries out the complementation only to homonous recombinant --] -- using -- Expand Long Template PCR system which used baffer1 The reacting (Loche) method was used. in addition, the program of the

PCR concerned -- a DNA danaturation (for 94 degrees C and 5 seconds) -- continuing -- denaturation (for 94-degree-C and 2 seconds)-annealing (for 55-degree-C and 2 seconds)-expanding (for 68 degrees C and 2 minutes) of 40 cycle -- it was the last expanding (for 68 degrees C and 5 minutes) further. In addition, in using the chromosome of a homonous recombination embryonic stem cell as mold, when using the chromosome of an un-homonous recombination embryonic stem cell (wild type embryonic stem cell) as mold for the DNA fragment of 2kbp(s), magnification of the DNA fragment of 0.5kbp(s) can be detected.
Thus, the genotype of the individual after F1 mouse with which the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight of the target mouse origin was destroyed in this example as a result of carrying out genotype analysis of a chimeric mouse was heterozygote (those of both DNA fragment of 0.5kbp, and DNA fragment of 2kbp(s) with magnification), or a wild type mouse (only a 0.5kbp fragment those with magnification). In addition, the incidence of heterozygote was moiety extent of the rate predicted.
[Example 7]
[0049]
(Adiposity acceleration protein gene expression check which are about 6000 or more molecular weight in an embryonic stem cell according to the PCR method or a RT-PCR analysis method to a mRNA extract list)
The mouse embryonic stem cell of a wild type and the mouse embryonic stem cell (embryonic stem cell to which the homonous recombination on the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight took place) by which frozen preservation was carried out in the example 3 were respectively homogenized in TRIzol RNA reagent (product made from GIBCO-BRL) independently, and all RNA was extracted according to the standard approach of the company. The quantum was carried out by carrying out the spectrometry of all the extracted RNA (melt) by UV spectrometer (260nm, 280nm).
The reverse transcription reaction was carried out using AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (product made from LIFE SCIENCES), and checked the existence and its amount of manifestations of the gene expression concerned in each embryonic stem cell by performing the PCR method or RT-PCR method on the conditions with which reflect it in the amount of adiposity acceleration protein gene expression which are about 6000 or more molecular weight contained, using obtained cDNA as mold, and magnification of a DNA fragment is accepted to be.
In this case, the used PCR method is KOD plus which uses the primer of the couple of primer GE-U7 (array number 14:GGC CAC AAA TTC CAG AGA ACA G) and primer GE-L7 (array number 15:CCA AAT GAG CAG ATG CCC CTA T). (the product made from Toyobo, and Japan) It was the reacting method. In addition, the program of the PCR concerned was the last expanding (for 68 degrees C and 5 minutes) at denaturation (for 94-degree-C and 10 seconds)-annealing (for 55-degree-C and 30 seconds)-expanding (for 68 degrees C and 1 minute) of 30 cycle, and a pan following the DNA danaturation (between 94 degrees C and 1 part). Consequently, said gene expression was checked in each embryonic stem

cell.
On the other hand, RT-PCR method was enforced using SYBR Green PCR Master MIX (ABI company make) which uses this primer, and analyzed the test result using PRISM 7900 HT Sequence Detection System 2.0 (ABI company make). Consequently, it was checked that said amount of gene expression is decreasing in abbreviation one half as compared with a wild type embryonic stem cell in the heterojunction mold embryonic stem cell of said genome gene.
[Example 8]
[0050]
(Check of the amount of adiposity acceleration protein gene expression which are about 6000 or more molecular weight in each organization by the RT-PCR analysis method)
The mouse organization of a wild type and the mouse organization (organization which extracted from the individual (about 18 weeks old) of the descendant animal after F1 invented from the embryonic stem cell to which the homonous recombination on the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight took place) of a heterojunction mold were respectively homogenized in TRIzol RNA reagent (product made from GIBCO-BRL) independently, and all RNA was extracted according to the standard approach of the company. The amount of gene expression concerned in various organizations was checked by performing RT-PCR method according to the approach indicated in the example 7 using all extracted RNA (melt).
Consequently, in various kinds of fat tissues (perimeter [testis] fat tissue, postabdomen film fat tissue, mesentery fat tissue, a subcutaneous adipose tissue, brown adipose tissue) of a heterojunction mold mouse individual, and other organizations (a blood vessel, liver, muscular system), it was checked as compared with this organization of a wild type mouse individual that the amount of adiposity acceleration protein gene expression which are about 6000 or more molecular weight is decreasing (table 1 reference). In addition, the amount of gene expression concerned in the various organizations in a table 1 is the value amended on the basis of the amount of manifestations of a mouse 36 B4 gene (acidic ribosomal phosphoprotein PO (Arbp)).
[0051]
[A table 1]

	脂肪組織					その他の組織		
マウス個体種	精巣周囲	後腹膜	腸間膜	皮下	褐色	血管	肝臓	筋肉
ヘテロ接合型マウス-雄	22.3	16.3	23.3	32.2	127.1	124.8	19.2	55.8
ヘテロ接合型マウス-雌	87.7	86.2	24.3	49.4	407.5	376.9	76.3	98.2
野生型マウス-雄	235.4	122.9	879.2	279.7	755.9	537.7	74.6	221.5
野生型マウス-雌	194.9	138.8	170.6	90.2	669.9	494.5	188.2	156.3

[Example 9]
[0052]
(Symptoms observation and analysis under a DIO (diet nature obesity induction) test condition)
1) Each was usually divided into the diet group (Std diet group), and a high fat and a high sucrose diet group (HH diet group) so that the weight average might become comparable, and general diet (D12328 (product made from Research Diets)) or a high fat food (D12331 (product made from Research Diets)) was made to take in freely for about 30 weeks from about 8 weeks old or subsequent ones about the male heterojunction mold mouse individual and male wild type mouse individual of a descendant animal after F1 invented from the embryonic stem cell.
2) The mouse was bred and condition observation and an autopsy (wood conversion) were performed. The environmental condition at the time of breeding carried out individual breeding within the 6 ream cage (W:750xD:100xH:100mm) made from stainless steel at the breeding room maintained by 12 air change rates/o'clock for the room temperature of 20-26 degrees C, 40 - 70% of humidity, and light-and-darkness 12 hours each (lighting: 6:00a.m. - 6:00 p.m.). The group reason of the mouse of a monograph affair was shown in a table 2.
[0053]
[A table 2]

群		食餌条件	性別	動物匹数
1.	ヘテロ接合型	普通食（Std食）	♂	7
2.	野生型		♂	7
3.	ヘテロ接合型	高脂肪・高ショ糖食（HH食）	♂	7
4.	野生型		♂	7

[0054]
3) A symptoms observation test method and a result are shown below.
(1) It carried out once during the diet load period about measurement of body weight at two weeks.
(2) Measurement after a 5-hour fast was once carried out during the diet load period about blood sugar level measurement at two weeks. Moreover, measurement after a 24-hour fast was carried out before a diet load (8 weeks old) and about the 27-week diet load back (37 weeks old). About 50microL blood was extracted using the heparinized capillary tube from the eye socket venous plexus of an animal which abstained from food under the water intake on the blood collecting day, and the blood sugar level of whole blood was measured in oxygen electrode method using the ANTO sense II (Daikin Industries, LTD.).
(Result)
It was [in / especially / the above result, a heterojunction mold mouse group is not based on the existence of diet nature obesity induction, but has the inclination for the blood sugar level to be high, as compared with a wild type mouse group, and / HH diet group's 24 hour fast conditions] significant hyperglycemia (drawing 3).
[0055]
4) About the glucose tolerance test, it carried out by the following approaches.
(1) Carbohydrate tolerance test (OGTT) : after carrying out a diet load for a long period of time (about 25 weeks), the glucose solution (1g [kg] /, 10 mL/kg) was administered orally to the animal which abstained from food under the water intake from the previous day for 18 to 24 hours (disposable syringe made from polypropylene equipped with a disposable metallicity sound). The blood sugar level of whole blood was measured by the approach of the above (2) before glucose administration and after [20 and 60] glucose administration and 120 minutes. In addition, the area under the curve value (AUC (area under the curve)) was computed from the blood sugar level in each measuring time.
(2) Insulin loading test (ipITT) : spacing was set and carried out about two weeks after carrying out Above OGTT. Intraperitoneal administration (disposable syringe made from polypropylene equipped with a hypodermic needle) of the insulin

solution (0.35 U/kg, 10 mL/kg) was carried out to the animal which abstained from food under the water intake from the previous day for 18 to 24 hours. The blood sugar level was measured by the approach of the above −1 just before an insulin load and after 30, 60, and 120 minutes. In addition, the area under the curve value (AUC (area under the curve)) was computed from the blood sugar level in each measuring time.

(Result)

In OGTT, the heterojunction mold mouse group was hyperglycemia significant after [of a Std foods breeding group] glucose administration 60 minutes, and after [of HH foods breeding group] glucose administration 120 minutes as compared with the wild type mouse group (table 3). Moreover, when ipITT was carried out and having been compared similarly, hyperglycemia was accepted after [of a Std foods breeding group] glucose administration 30,120 minutes (table 4). It was not based on the existence of diet nature obesity induction from these results, but, as for the heterojunction mold mouse group, insulin susceptibility (glucose tolerance) was falling.

[0056]

[A table 3]

	経口投与後経過時間(分)			
	0	20	60	120
Std食hetero	147.3[a]	310.6	235.1[b]	218.7
Std食wild	117.6	300.9	202.4	182.7
HH食hetero	171.4[c]	356.9	252.1	214.1[d]
HH食wild	145.4	311.7	214.3	178.4

a:P<0.055 投与前のStd食hetero/wild群間
b:P<0.074 投与60分後のStd食hetero/wild群間
c:P<0.057 投与前のHH食hetero/wild群間
d:P<0.044 投与120分後のHH食hetero/wild群間

[0057]

[A table 4]

	インスリン腹腔内投与後経過時間(分)			
	0	30	60	120
Std食hetero	143.3[a]	128.1[b]	111.7	131.0[c]
Std食wild	118.6	90.1	83.9	82.6
HH食hetero	178.9[d]	136.6	124.7	133.6
HH食wild	155.4	108.6	98.6	106.4

a:P<0.1 投与前のStd食hetero/wild群間
b:P<0.067 投与30分後のStd食hetero/wild群間
c:P<0.06 投与120分後のStd食hetero/wild群間
d:P<0.042 投与前のHH食hetero/wild群間

[0058]
5) autopsy: -- above-mentioned 4- after carrying out 1 and 2, it dissected by setting spacing about two weeks. It carried out the exsanguination (about 0.6 to 0.8 mL) from the abdominal aorta under pentobarbital (30 mg/mL, 2 mL/kg) anesthesia after the about 5-hour fast under the water intake. The blood sugar level was measured by the approach of 3 (2) using the extracted blood (whole blood), and after it carried out centrifugal separation (for 14-20 degrees C, 3000rpm, and 15 minutes) of the remaining blood within 30 minutes after blood collecting and it separated the blood serum and the clot, it carried out distributive-pouring / freezing (-80 degrees C) preservation of the blood serum part at the ice-cooling tube. Liver, the kidney, a mesentery fat, a perimeter [testis] fat, skinfold thickness, regions-of-back brown fat, a thorax main artery, right-hand side femoral region muscles, a testis or the ovary, a uterus, and the pancreas were extracted after blood collecting. The actuation after extraction is indicated below.
Liver, a mesentery fat, and regions-of-back brown fat fixed the part with liquid nitrogen after the gravimetry, and fixed freezing and the remainder with neutral buffered formalin 10%. A perimeter [testis] fat, skinfold thickness, and the kidney were similarly frozen after the gravimetry. The thorax main artery and the reproductive organ were frozen similarly. Femoral region muscles froze the part similarly and fixed the remainder with formalin. The pancreas were fixed with formalin.
[0059]
6) The blood serum which extracted and carried out frozen preservation from the wild type mouse and the heterojunction mold mouse with the analysis above 5 of a

blood serum parameter is glucose concentration (Glu (mg / 100ml)), total cholesterol concentration (T-Cho (mg / 100ml)), HDL cholesterol (HDL-Cho (mg / 100ml)), and phospholipid (measurement of PL (mg / 100ml) was presented.). : Consequently, it was not concerned with Std foods and HH foods conditions, but, as for the heterojunction mold mouse group, the amount of blood cholesterol levels (T-Cho, HDL-Cho) and the phospholipid value were rising as compared with the wild type mouse group (drawing 4 , drawing 5 , and drawing 6).

[0060]

7) each organization by the RT-PCR analysis method (per 2microg unit) the check of the amount of adiposity acceleration protein gene expression express with the array number 1 which can be set be bred over a long period of time under the Std foods which carried out wood conversion and which carried out frozen preservation by 4 , or HH foods load conditions . it carried out by the same approach as an example 8 using the organization of about 38 - weeks old wild type mouse group and a heterojunction mold mouse group .

Consequently, the gene expression of the adiposity promoter expressed with the array number 1 was the highest in brown adipose tissue, and genotype was reflected, i.e., as compared with the wild type, the amount of manifestations was one half mostly. It was what the amount of manifestations in liver is notably low, and is about 1 of the amount of manifestations in brown adipose tissue / 30 to 50 times, and does not reflect genotype on the other hand. Moreover, by the mesentery fat tissue and liver of a wild type mouse group, the significant increment in a manifestation was accepted with HH foods load. There was no effect by the diet load in brown fat (table 5).

[0061]

[A table 5]

	組織(全RNA 2μg)の 発現レベル			
	褐色脂肪	腸管膜脂肪	精巣周辺脂肪	肝臓
Std食 hetero	254.7	23.8	43.7	8.3
Std食 wild	478.9	25.1	61.7	8.0
HH食 hetero	227.8	18.7	26.6	24.4
HH食 wild	414.8	46.1	47.4	19.6

[Example 10]

[0062]

(Neutral fat accumulated dose measurement in liver)

About the neutral fat extract from liver tissue, by 5 of an example 9, a wild type mouse, Wood conversion is carried out and about 0.2g (an actual measurement is set to Xg) of the liver which carried out frozen preservation is cut out from a heterojunction mold mouse. And within 1.5ml PBS (phosphate buffered saline and GIBCO) It homogenized using the poly TRON (PT10made from KINEMATICA /35

molds) independently respectively (for [output memory] 7 or 10 seconds), and the 10ml chloroform methanol (2:1) was added to this, and it stirred violently to it. After leaving it at 4 more degrees C for 2 hours or more, centrifugal separation (for 2500rpm and 10 minutes) was carried out, and the solid layer which came floating was removed. 3ml added to this, MgCl2 of 4mM(s) was stirred to it, although centrifugal separation (for 2500rpm and 10 minutes) was carried out after neglect for 30 minutes at the room temperature, the capacity of lower layer liquid was read (an actual measurement is set to Yml), among these 0.1ml was isolated preparatively to EPPEN. The solvent of the lower layer liquid isolated preparatively was volatilized in the speed back, and 0.2ml isopropanol was added to the solute which remained, it dissolved in it, and this was made into the neutral fat extract. Although carried out according to the standard approach of the company using triglyceride-Test Wako (Wako Pure Chem make) about measurement of the amount of neutral fat contained in a neutral fat extract, the rough approach of the capacity in the case of making the extract from liver into a specimen and the amount of neutral fat is described below.

After repeating the actuation which puts the adsorbent of about one cup of earpick on 0.2ml of neutral fat extracts gently for 5 minutes after stirring during addition and 5 seconds 3 times, it isolated preparatively to EPPEN with the supernatant liquid new 0.1ml which carried out centrifugal separation (for a room temperature, 10,000rpm, and 5 minutes). 45microl addition of KOH is done 5% at the neutral fat extract and the criteria liquid dilution train for calibration-curve creation (0-200 mg/dl, 0.1ml each) which were isolated preparatively. For 5 seconds, after stirring, put and rank second for 15 minutes at 37 degrees C, and 0.1ml of buffer solutions and the mixed liquor of 0.01ml of oxidizers are added. For 5 seconds, after stirring, at 37 degrees C, standing and after 0.2ml's having added and putting a color reagent for 40 minutes at 37 degrees C after stirring for 5 seconds further, it cooled for 3 minutes and the coloring reaction was stopped for 15 minutes. 0.2ml of the sample after a coloring reaction was isolated preparatively on the coaster 96 hole plate (black), and the absorbance of 405nm was measured (an actual measurement is set to OD405nm). The calibration curve was created from the absorbance (OD405nm) of the criteria liquid dilution train sample after a reaction, and the amount of neutral fat in a neutral fat extract was converted (a reduced property is made into Zmg/dl). Amount of neutral fat (mg/g organization) =2YZ/100X contained in each liver tissue were convertible from the actual measurement of Above X, Y, and Z. However, it measured by adjusting so that it may correspond to this system by diluting in the phase of a neutral fat extract to about about 16 times about a remarkable sample with much neutral fat.

Consequently, comparing with Std foods breeding about a wild type mouse group, under HH foods breeding conditions, the amount of neutral fat in liver increased twice [about], and it increased by about 3 times about the heterojunction mold mouse group (drawing 7).

That is, in the bottom of a meal nature obesity induction condition, as compared with the wild type, are recording of liver neutral fat was increasing intentionally the heterojunction mold mouse of the adiposity acceleration protein which is about

6000 or more molecular weight by 1.6 times, and sthenia of fatty liver Mr. symptoms was accepted.

[Example 11]

[0063]

(Manifestation level analysis of the symptoms related factor in each organization by the RT-PCR analysis method)

Each organization (per 2microg unit) Manifestation level analysis of the symptoms related factor which can be set was carried out by the same approach as an example 10-7. What was indicated by the table 6 was used as a primer for PCR.

[0064]

[A table 6]

因子名	5' 側プライマー	配列番号	3' 側プライマー	配列番号
PEPCK	gagatagcggcacaat	１６	ttcagagactatgcggtgat	１７
G6P	cgccatgcaaaggactagga	１８	gcctgggcttgctcttctgtatctg	１９
UCP1	cgggtcctggaacgtcatca	２０	ttctttggttggttttattcgtggt	２１
FAS	gctggcattcgtgatggagtcgt	２２	aggccaccagtgatgatgtaactct	２３
ACO	tgacctgccgagccagcgtat	２４	gacagaagtcaagttccacgccact	２５

(Result)

In mesentery fat tissue, it compared with Std foods and HH foods breeding wild type mouse group, and significant manifestation sthenia of ACO (acyl-CoA oxidase) was accepted by the heterojunction mold mouse group (drawing 8). This was what disagrees with the genotype of the adiposity acceleration protein which is about 6000 or more molecular weight. The pattern (drawing 9) with the same said of the manifestation in a perimeter [testis] fat was shown.

Moreover, in liver, it is not concerned with diet conditions by the heterojunction mold mouse group. Manifestation sthenia of PEPCK (phosphoenolpyruvate carboxykinase) of liver was accepted (drawing 10). Moreover, under Std foods breeding conditions, manifestation sthenia of G6P (glucose-6-phosphatase) was accepted (drawing 10).

further -- brown adipose tissue -- diet conditions -- not being concerned -- UCP1 (uncoupling protein 1) -- being FAS (fatty acid synthase) -- significant manifestation sthenia was accepted (drawing 11). These were what disagrees with the genotype of the adiposity acceleration protein which is about 6000 or more molecular weight.

[0065]

[The statistical approach]

In each above-mentioned example, statistical analysis computed the average **

standard error for every group, significant difference assay was performed about Std foods or HH foods using the commercial statistics program by the 2 between groups of a heterojunction mold mouse group and a wild type group, and the value of a t test was written together only about less than (P< 0.10) 10%.

[Brief Description of the Drawings]

[0066]

[Drawing 1] The inside of the DNA fragment of the genome gene of the adiposity acceleration protein whose drawing 1 is about 6000 or more molecular weight of construction of a targeting vector, and the mouse origin, The homonous recombination approach of replacing the DNA fragment which has a base sequence equivalent to the field from an exon 3 to an exon 4 The gestalt of (the homonous recombination approach [namely,] by which DNA which consists of an exon 2-SA-IRIS-beta geo-pA gene cassette-exon 6 which said targeting vector has is permuted) It is drawing having shown (namely, Targeting Vector which replaced with the beta-geo cassette of the pGT1.8IresBgeo origin the exon 3-4 which carries out cloning of the exon 2-6 to pCR2.1, sets to pCR-085, and is started to a MunI site). Five small boxes on the thick wire showing the genome gene of the adiposity acceleration protein which is about 6000 or more molecular weight [topmost part] of the mouse origin show the exon. Three small boxes on the thick wire showing the targeting vector of an inside step show the exon. Moreover, the oblong box in a center section is a SA-IRIS-beta geo-pA gene cassette (here). SA of the mouse EN2 origin (splicing accepter), IRES of the encephalomyocarditisecond Virus origin (internal ribosome entry site), DNA which comes to process pA (polyadenylation signal) of the SV40 origin to betageo with which it makes it come to unite beta-galactocidase and a neomycin resistance gene flush end-ization (a SA-IRIS-beta geo-pA gene cassette is meant.) It is shown. The oblong box which three small boxes on the thick wire showing the lowermost genome gene of a homonous recombination homozygote show the exon, and is located in the center section shows the SA-IRIS-beta geo-pA gene cassette. Exon 2 which said targeting vector has by such homonous recombination on an original promotor's lower stream of a river which the genome gene concerned originally has - DNA which consists of said SA-IRIS-beta geo-pA gene cassette-exon 6 is permuted, and the structural protein coding region which is equivalent to the field to an exon 3 to a part of exon 4 as a result carries out deletion. Since the codon of an exon 2 is not in agreement with the amino-acid-residue frame of the continuing structural gene (a gene cassette or exon 4) at this time, the protein produced does not form fusant with the adiposity acceleration protein which is about 6000 or more molecular weight of the mouse origin.

[Drawing 2] It is drawing having shown the result of Southern-blotting analysis of the genome gene which a homonous recombination embryonic stem cell has. On a lane 1, the detection band of a size marker (10, 8, 6, 4, 3, 2, 1.5 kb) and lanes 2, 3, 4, and 6 can check the detection band of a wild type (only 16kbp), and can check the detection (two kinds) band of a heterojunction mold (10kbp and 16kbp(s)) on a lane 5.

[Drawing 3] It is drawing showing the blood sugar level. Std_H expresses among drawing the heterojunction mold mouse which gave general diet, Std_W expresses the wild type mouse which gave general diet, HH_H expresses the heterojunction mold mouse which gave HH foods, and HH_W expresses the wild type mouse which gave HH foods. After making the blood sugar level abstain from food after a diet load for 24 hours, it was measured.
[Drawing 4] It is drawing showing the amount of total cholesterol in a blood serum. The semantics of the legend in a graph is the same as drawing 3 .
[Drawing 5] It is drawing showing the amount of HDL cholesterol in a blood serum. The semantics of the legend in a graph is the same as drawing 3 .
[Drawing 6] It is drawing showing the amount of phospholipid in a blood serum. The semantics of the legend in a graph is the same as drawing 3 .
[Drawing 7] the TORIGURISE resaler in liver tissue -- it is drawing showing the amount of id (TG). The semantics of the legend in a graph is the same as drawing 3 .
[Drawing 8] It is drawing showing the glycolipid metabolic turnover related factor in mesentery fat tissue, and the amount of manifestations of ACO. The semantics of the X-axis in a graph is the same as drawing 3 .
[Drawing 9] It is drawing showing the glycolipid metabolic turnover related factor in perimeter [testis] fat tissue, and the amount of manifestations of ACO. The semantics of the X-axis in a graph is the same as drawing 3 .
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[Drawing 11] It is drawing showing the glycolipid metabolic turnover related factor in brown adipose tissue, and the amount of manifestations of FAS and UCP1. The semantics of the X-axis in a graph is the same as drawing 3 .
[Array table free text]
[0067]
Array number 3
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 4
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 5
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 6
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 7
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 8
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 9
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 10
Oligo NUKURETOCHIDO which is the primer designed for PCR

Array number 11
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 12
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 13
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 14
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 15
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Array number 24
Oligo NUKURETOCHIDO which is the primer designed for PCR
Array number 25
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[Translation done.]

DESCRIPTION OF DRAWINGS

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